

ULTRASOUND STUDIES OF DILATED CARDIOMYOPATHY  
IN DOBERMANNS AND ENGLISH COCKER SPANIELS.

VIRGINIA LUIS FUENTES

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## Declaration

Carmel Moran provided assistance with the integrated backscatter recordings, and Helen Brown performed the statistical analysis included in the paper published from this thesis in the American Journal of Veterinary Research. This is included as Appendix 4. Two abstracts have been published; one in the Journal of the American Society of Echocardiography, and one in the British Heart Journal. The rest of this thesis is my own work and has not been presented to any university other than the University of Edinburgh.

Virginia Luis Fuentes

May 2000



To my parents, with love.

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# CONTENTS

<b>ABBREVIATIONS.....</b>	<b>7</b>
<b>ABSTRACT.....</b>	<b>10</b>
<b>CHAPTER 1: REVIEW OF THE LITERATURE.....</b>	<b>12</b>
<b>1.1 DILATED CARDIOMYOPATHY .....</b>	<b>12</b>
1.1.1 <i>Classification of Cardiomyopathies</i>	12
1.1.2 <i>Aetiology of dilated cardiomyopathy</i>	13
1.1.3 <i>Animal models of DCM</i>	13
1.1.4 <i>Naturally occurring DCM in animals</i>	14
1.1.5 <i>Canine DCM</i>	14
1.1.6 <i>Pathological findings in canine DCM</i>	16
1.1.7 <i>Clinical features of canine DCM</i>	18
1.1.8 <i>History and physical findings</i>	20
1.1.9 <i>Ancillary diagnostic tests</i>	21
1.1.10 <i>Treatment of canine DCM</i>	21
<b>1.2 M-MODE ECHOCARDIOGRAPHY.....</b>	<b>23</b>
1.2.1 <i>History of M-mode echocardiography</i>	23
1.2.2 <i>M-mode measurement and reproducibility</i>	24
1.2.3 <i>M-mode echocardiography in the assessment of cardiac dimensions</i>	28
1.2.4 <i>M-mode echocardiography and LV volume measurement</i>	28
1.2.5 <i>M-mode echocardiographic assessment of ventricular function</i>	29
1.2.6 <i>Assessment of left atrial size</i>	32
1.2.7 <i>M-mode echocardiography in dogs</i>	33
<b>1.3 QUANTITATIVE TWO-DIMENSIONAL ECHOCARDIOGRAPHY .....</b>	<b>38</b>
1.3.1 <i>2DE and the assessment of cardiac volumes</i>	39
1.3.2 <i>2DE assessment of left atrial size</i>	48
<b>1.4 DOPPLER ECHOCARDIOGRAPHY .....</b>	<b>50</b>
1.4.1 <i>Principles of Doppler echocardiography</i>	51
1.4.2 <i>Doppler echocardiography in the assessment of systolic function</i>	53
1.4.3 <i>Doppler echocardiography in the assessment of diastolic function</i>	56

1.4.4	<i>Doppler assessment of valvular regurgitation</i>	65
1.4.5	<i>Veterinary applications of Doppler echocardiography</i>	67
<b>1.5</b>	<b>ULTRASONIC TISSUE CHARACTERISATION .....</b>	<b>70</b>
1.5.1	<i>Interaction of ultrasound waves with biological tissue</i>	70
1.5.2	<i>Cyclic variation in integrated backscatter (CVIB)</i>	77
1.5.3	<i>IB findings in cardiac disease</i>	80

## CHAPTER 2: STUDY SUBJECTS ..... 83

<b>2.1</b>	<b>INTRODUCTION.....</b>	<b>83</b>
<b>2.2</b>	<b>BACKGROUND .....</b>	<b>83</b>
2.2.1	<i>Dobermann DCM</i>	83
2.2.2	<i>DCM in English Cocker spaniels</i>	91
<b>2.3</b>	<b>STUDY ANIMALS .....</b>	<b>96</b>
2.3.1	<i>Selection criteria</i>	96
<b>2.4</b>	<b>STATISTICAL METHODS .....</b>	<b>97</b>
<b>2.5</b>	<b>RESULTS .....</b>	<b>98</b>
2.5.1	<i>Normal dogs</i>	98
2.5.2	<i>Dobermanns with dilated cardiomyopathy</i>	99
2.5.3	<i>English cocker spaniels with dilated cardiomyopathy</i>	101
2.5.4	<i>Comparison of Breed Groups</i>	102
<b>2.6</b>	<b>DISCUSSION .....</b>	<b>106</b>
2.6.1	<i>Choice of breeds with DCM</i>	106
2.6.2	<i>Choice of normal control group</i>	106

## CHAPTER 3: EVALUATION OF VENTRICULAR FUNCTION IN NORMAL DOGS AND DOGS WITH DCM USING M-MODE ECHOCARDIOGRAPHY ..... 108

<b>3.1</b>	<b>AIMS .....</b>	<b>108</b>
<b>3.2</b>	<b>BACKGROUND .....</b>	<b>109</b>
3.2.1	<i>M-mode echocardiographic indices in human DCM</i>	109
3.2.2	<i>M-mode prognostic indicators in human DCM</i>	110
3.2.3	<i>M-mode assessment of canine DCM</i>	111
<b>3.3</b>	<b>MATERIALS AND METHODS .....</b>	<b>116</b>

3.3.1	<i>Equipment</i>	116
3.3.2	<i>Methods</i>	117
3.3.3	<i>Protocol</i>	118
3.3.4	<i>M-mode measurements</i>	118
3.3.5	<i>Statistical methods</i>	121
<b>3.4</b>	<b>RESULTS .....</b>	<b>122</b>
3.4.1	<i>German short-haired pointers</i>	122
3.4.2	<i>Dobermanns and cocker spaniels with DCM</i>	126
3.4.3	<i>Comparison of GSH pointers and dogs with DCM</i>	131
<b>3.5</b>	<b>DISCUSSION .....</b>	<b>136</b>
3.5.1	<i>Normal GSH pointers</i>	136
3.5.2	<i>Comparison of results in normal dogs with other studies</i>	138
3.5.3	<i>Cocker spaniels with DCM</i>	140
3.5.4	<i>Dobermanns with DCM</i>	141
3.5.5	<i>Comparison of normal dogs and dogs with DCM</i>	142
3.5.6	<i>Comparison of Dobermanns and cocker spaniels</i>	143
<b>3.6</b>	<b>CONCLUSIONS .....</b>	<b>145</b>

## CHAPTER 4: EVALUATION OF VENTRICULAR FUNCTION IN NORMAL DOGS AND DOGS WITH DCM USING TWO-DIMENSIONAL ECHOCARDIOGRAPHY ..... 146

<b>4.1</b>	<b>INTRODUCTION.....</b>	<b>146</b>
<b>4.2</b>	<b>AIMS .....</b>	<b>146</b>
<b>4.3</b>	<b>BACKGROUND .....</b>	<b>147</b>
4.3.1	<i>2D echocardiographic assessment of human DCM</i>	147
4.3.2	<i>2DE prognostic indicators in human DCM</i>	148
4.3.3	<i>2DE assessment of canine DCM</i>	149
<b>4.4</b>	<b>MATERIALS AND METHODS .....</b>	<b>149</b>
4.4.1	<i>Equipment</i>	149
4.4.2	<i>Methods</i>	151
4.4.3	<i>Protocol</i>	151
4.4.4	<i>2DE measurements</i>	152
4.4.5	<i>Statistical methods</i>	156
<b>4.5</b>	<b>RESULTS .....</b>	<b>156</b>
4.5.1	<i>German short-haired pointers</i>	156
4.5.2	<i>Dobermanns with DCM</i>	163

4.5.3	<i>Cocker Spaniels with DCM</i>	168
4.5.4	<i>Comparison of GSH pointers and dogs with DCM</i>	172
<b>4.6</b>	<b>DISCUSSION .....</b>	<b>179</b>
4.6.1	<i>Right parasternal long axis view and LV volumes in dogs</i>	179
4.6.2	<i>Comparison of 2DE and M-mode values</i>	181
4.6.3	<i>Comparison of normal dogs and dogs with DCM</i>	185
4.6.4	<i>Comparison of affected Dobermanns and cocker spaniels</i>	186
<b>4.7</b>	<b>CONCLUSIONS .....</b>	<b>187</b>

## CHAPTER 5: EVALUATION OF VENTRICULAR FUNCTION IN NORMAL DOGS AND DOGS WITH DCM USING DOPPLER ECHOCARDIOGRAPHY ..... 189

<b>5.1</b>	<b>INTRODUCTION.....</b>	<b>189</b>
<b>5.2</b>	<b>AIMS .....</b>	<b>189</b>
<b>5.3</b>	<b>BACKGROUND .....</b>	<b>190</b>
5.3.1	<i>Doppler-derived systolic indices in human DCM</i>	190
5.3.2	<i>Doppler-derived diastolic indices in human DCM</i>	191
5.3.3	<i>Doppler echocardiographic prognostic indicators</i>	194
5.3.4	<i>Assessment of canine DCM with Doppler echocardiography</i>	196
<b>5.4</b>	<b>MATERIALS AND METHODS .....</b>	<b>198</b>
5.4.1	<i>Equipment</i>	198
5.4.2	<i>Methods</i>	200
5.4.3	<i>Protocol</i>	201
5.4.4	<i>Measurements of Doppler echocardiographic variables</i>	202
5.4.5	<i>Statistical methods</i>	206
<b>5.5</b>	<b>RESULTS .....</b>	<b>206</b>
5.5.1	<i>German short-haired pointers</i>	206
	<i>Mitral</i>	210
	<i>PEP/ET</i>	211
5.5.2	<i>Dobermanns with DCM</i>	214
5.5.3	<i>Cocker spaniels with DCM</i>	216
5.5.4	<i>Comparison of GSH pointers and dogs with DCM</i>	219
<b>5.6</b>	<b>DISCUSSION .....</b>	<b>232</b>
5.6.1	<i>Normal GSH pointers</i>	233
5.6.2	<i>Reproducibility</i>	236

5.6.3	<i>Dobermanns and cockers spaniels with DCM</i>	238
5.6.4	<i>Further Doppler applications</i>	248
5.7	<b>CONCLUSIONS .....</b>	<b>248</b>

## CHAPTER 6: INTEGRATED BACKSCATTER MEASUREMENT IN NORMAL DOGS AND DOGS WITH DCM250

6.1	<b>INTRODUCTION.....</b>	<b>250</b>
6.2	<b>AIMS .....</b>	<b>250</b>
6.3	<b>BACKGROUND .....</b>	<b>251</b>
6.3.1	<i>IB studies in human DCM</i>	251
6.4	<b>MATERIALS AND METHODS .....</b>	<b>253</b>
6.4.1	<i>Animals</i>	253
6.4.2	<i>Imaging system and RF data acquisition</i>	253
6.4.3	<i>Protocol</i>	258
6.4.4	<i>Analysis of data</i>	259
6.5	<b>RESULTS .....</b>	<b>260</b>
6.5.1	<i>Normal dogs</i>	260
6.5.2	<i>Dogs with DCM</i>	263
6.5.3	<i>Comparison between normal dogs and dogs with DCM</i>	267
6.6	<b>DISCUSSION .....</b>	<b>269</b>
6.6.1	<i>Study protocol</i>	269
6.6.2	<i>Normal dogs</i>	270
6.6.3	<i>Dogs with DCM</i>	275
6.7	<b>CONCLUSIONS .....</b>	<b>277</b>

## CHAPTER 7: GENERAL DISCUSSION .....279

7.1	<b>STUDY LIMITATIONS .....</b>	<b>281</b>
7.2	<b>FURTHER STUDIES.....</b>	<b>282</b>

APPENDIX 1.....	284
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MODIFIED NEW YORK HEART ASSOCIATION (NYHA) CLASSIFICATION .....	284
APPENDIX 2.....	285
VOLUME MEASUREMENTS USING AREA-LENGTH METHOD	285
APPENDIX 3.....	289
AORTIC MEASUREMENTS FROM SUBCOSTAL VIEW.....	289
REFERENCES .....	290



## ABBREVIATIONS

2DE	two-dimensional echocardiography
A	atrial filling
ab	abnormal relaxation
acc dt	acceleration time
ACE	angiotensin converting enzyme
AF	atrial fibrillation
ANOVA	analysis of variance
Ao	aorta, aortic
AoV	aortic valve
APC	atrial premature complex
ASE	American Society of Echocardiography
AT	acceleration time
bpm	beats per minute
BSA	body surface area
CHF	congestive heart failure
CSp	English cocker spaniel
CV%	coefficient of variation
CV <sub>ED-ES</sub>	cyclic variation in IB between end-diastole and end-systole
CVIB	cyclic variation in integrated backscatter
CV <sub>max-min</sub>	cyclic variation in IB between maximum and minimum values
CW	continuous wave
dB	decibel
DCM	dilated cardiomyopathy
DE	Doppler echocardiography
decel	deceleration
Dob	Dobermann
Dobes	Dobermanns
dP	change in pressure
dP/dt	rate of change of pressure rise
DT	deceleration time
dt	increment in time
dur	duration
dV/dt max	maximum rate of acceleration
dV/dt	acceleration
E DT	early filling deceleration time
E	early filling
E/A	ratio of early filling to atrial filling
ECG	electrocardiogram
EDV	left ventricular end-diastolic volume
EDVI	left ventricular end-diastolic volume index

EPSS	mitral E-point to septal separation
ESV	left ventricular end-systolic volume
ESVI	left ventricular end-systolic volume index
ET	ejection time
Euth	euthanased
F	female
FN	neutered female
FS%	left ventricular fractional shortening
G Ret	golden retriever
GSH	German short-haired
GSP	German short-haired pointer
HCM	hypertrophic cardiomyopathy
HPRF	high pulse repetition frequency
HR	heart rate
IB	integrated backscatter
IVRT	isovolumic relaxation period
IVSd	interventricular septal thickness in diastole
IVSs	interventricular septal thickness in systole
IWH	Irish wolfhound
L	left
LA	left atrium, left atrial
LAp	left apical
LV	left ventricle, left ventricular
LV dP/dt max	maximum rate of change of left ventricular pressure
LVDd	left ventricular diameter in diastole
LVDs	left ventricular diameter in systole
LVET	left ventricular ejection time
LVPWd	left ventricular free wall thickness in diastole
LVPWs	left ventricular free wall thickness in systole
MB	Megabyte
MEA	mean electrical axis
MHz	MegaHertz
mmHg	millimeters of mercury
MN	neutered male
MR	mitral regurgitation
MV	mitral valve
n/a	not applicable
Newf	Newfoundland
No.	number
NR	not recorded
NS	not significant

NYHA	New York Heart Association
P(t)	amplitude of radiofrequency signal from phantom
PA	pulsus alternans, pulmonary artery
PEP	pre-ejection period
Pim	pimobendan
PW	pulsed wave
PW1	region of interest in basal LV free wall
PW2	region of interest in apical LV free wall
PWth%	percentage free wall thickening
R	right
Rad/thD	ratio of left ventricular radius to free wall thickness in diastole
Rad/thS	ratio of left ventricular radius to free wall thickness in systole
res	restrictive
RF	radiofrequency
S3	third heart sound
SC	subcostal
SD	standard deviation
SP	region of interest in interventricular septum
SR	sinus rhythm
TR	tricuspid regurgitation
TV	tricuspid valve
US	United States of America
V(t)	amplitude of RF signal in myocardial region of interest
Vcf	velocity of circumferential fibre shortening
vel	velocity
VPC	ventricular premature complex
vs	versus
VTI	velocity time integral
$\tau$	tau, time constant of isovolumic relaxation

## ABSTRACT

The basic hypothesis tested in this study was that dogs with dilated cardiomyopathy have abnormal ventricular function, and that this can be detected noninvasively using M-mode, two-dimensional (2D), and Doppler echocardiography, and also by measuring myocardial ultrasonic integrated backscatter. An additional hypothesis was that different breeds of dogs affected with DCM have a different clinical course, and that differences in ventricular function between these breeds can be detected by echocardiography or integrated backscatter measurements that might account for the different prognosis.

The aims of this study therefore were (1) to identify echocardiographic variables that distinguished normal dogs from dogs with DCM; (2) to identify differences in ventricular function using echocardiography between Dobermanns and English cocker spaniels with DCM; and (3) to measure ultrasonic integrated backscatter in normal dogs and in Dobermanns and Cocker spaniels with DCM.

In this group of Dobermanns, median survival time was 98 days (range 16 - 508 days), whereas median survival in the cocker spaniels was 512 days (range 51 to >1388 days), with 6/11 still alive at the time of writing ( $p < 0.002$ ). All the measured M-mode variables differed significantly between the normal dogs and the dogs with DCM. Compared with the Dobermanns, the cocker spaniels had significantly increased LV free wall thickening and increased LV diastolic diameter when indexed to body surface area. All the 2D echocardiographic variables were significantly different between the normal dogs and dogs with DCM when corrected for body size, but there were no significant differences between Dobermanns and cocker spaniels. Significant differences were found between normal and DCM dogs in some but not all of the Doppler echocardiographic variables. Cocker spaniels had higher mitral A wave velocities than the normal dogs, and decreased

mitral E/A ratios compared with both the other groups. Dobermanns had shorter isovolumic relaxation times than the other groups.

In the second part of this study, measurements were made of ultrasonic integrated backscatter in selected regions of the left ventricle in normal dogs and dogs with DCM. Ultrasonic integrated backscatter is a measurement of the power of the ultrasound signal returned from the myocardium to the transducer. This backscattered signal reflects basic tissue properties, and exhibits dynamic variation with contractile function that may represent fundamental alterations in the shape, size and distribution of scatterers within the myocardium. Consistent cyclic variation in integrated backscatter was observed in the LV free wall and septum of the normal dogs, but this pattern was not consistently seen in the DCM dogs. Differences were not seen between the Dobermanns and cocker spaniels, although the number of cocker spaniels measured was small.

In conclusion, the DCM dogs had evidence of markedly reduced systolic function compared with the normal dogs. The affected dogs had left atrial and left ventricular dilation with thinner left ventricular walls. Although there was little evidence of any difference in systolic function between the two affected breeds, there was evidence of different diastolic function, with a tendency for Dobermanns to show a restrictive pattern of transmitral filling, and cocker spaniels to show evidence of delayed relaxation. The different pattern of diastolic dysfunction in the two breeds is consistent with the prognostic value ascribed to transmitral flow patterns in human DCM patients: delayed relaxation patterns have been associated with improved survival times in man. Reduced cyclic variation of ultrasonic integrated backscatter was also seen in the dogs affected with DCM, although no differences were found between the two affected breeds.

## CHAPTER 1: REVIEW OF THE LITERATURE

### 1.1 DILATED CARDIOMYOPATHY

#### 1.1.1 Classification of Cardiomyopathies

The cardiomyopathies have been defined as a group of diseases of unknown aetiology that affect the myocardium, distinct from “specific heart muscle disease” (WHO/ISFC task force, 1980). This system of classification was recently revised, so that the term “cardiomyopathy” no longer excludes conditions where the aetiology is known. Specific heart muscle diseases are now classified as “specific cardiomyopathies” and defined in terms of their cause, such as ischaemic cardiomyopathy, or hypertensive cardiomyopathy (Richardson *et al.*, 1996). The revised World Health Organisation (WHO) classification of the three basic types is based on morphology and function where an underlying cause is not known. The *dilated* form is characterised by chamber dilation and predominantly contractile dysfunction; the *hypertrophic* form is characterised by inappropriate hypertrophy and diastolic dysfunction; and a *restrictive* form has diastolic dysfunction as the principle abnormality, often associated with abnormal infiltration of the myocardium. These three forms of primary myocardial disease may overlap with some of the specific cardiomyopathies. For example, alcohol abuse can lead to a form of myocardial disease that is indistinguishable from that of idiopathic dilated cardiomyopathy (McKenna *et al.*, 1998). Other terms currently used in place of “idiopathic dilated cardiomyopathy” include “nonischaemic cardiomyopathy”, or even “initially unexplained cardiomyopathy” (Felker *et al.*, 2000).

### 1.1.2 Aetiology of dilated cardiomyopathy

There have been many suggested aetiologies for “idiopathic” DCM, including viral infection (Woodroof, 1980), and autoimmunity (Schulteiss and Bolte, 1985). As more and more instances of idiopathic dilated cardiomyopathy are reclassified as specific cardiomyopathies, research has intensified on a search for underlying mechanisms, with particular interest in familial or genetic forms (Durand *et al.*, 1995; Marin-Garcia *et al.*, 1996; Michels *et al.*, 1992; Schmidt *et al.*, 1988; Zachara *et al.*, 1993). Although it seems likely that dilated cardiomyopathy represents a common end-point for a variety of different aetiopathological processes, progress has been made in identifying specific gene mutations that result in dilated cardiomyopathy in humans (Graham and Owens, 1999).

### 1.1.3 Animal models of DCM

Excluding animal models of left ventricular dysfunction induced by ischaemia and infarction, the most common method of inducing left ventricular failure has probably been that of rapid ventricular pacing, where systolic dysfunction evolves over time as a result of neurohormonal and myocardial changes induced by tachycardia (Elsner and Riegger, 1995b; Moe and Armstrong, 1999). Doxorubicin toxicity also results in global myocardial damage that can mimic the results of DCM (Elsner and Riegger, 1995a; Gwathmey and Davidoff, 1994), as can cobalt toxicity (Sandusky *et al.*, 1981). Cardiomyopathy in the Syrian hamster is another widely used model (Stauch and Lossnitzer, 1975).

A number of transgenic mouse models are being created with specific molecular deficiencies produced by gene targeting, which result in DCM-like syndromes (Kadambi and Kranias, 1998; Liu *et al.*, 1995). A phenotype resembling DCM in mice has been shown to result from abnormalities in cytoskeletal proteins (Arber *et al.*, 1997) and cytokines (Kubota *et al.*, 1997), disturbances in calcium handling, and abnormalities of a variety of intracellular signal transduction pathways (Cho *et al.*, 1999; Mende *et al.*, 1999; Sussman *et al.*, 1999).

#### 1.1.4 Naturally occurring DCM in animals

A dietary taurine deficiency was found to be responsible for many cases of dilated cardiomyopathy in cats, a condition that was previously assumed to be idiopathic in this species (Pion *et al.*, 1987). Since pet food manufacturers changed the formulation of proprietary cat foods, DCM is now seen only sporadically in cats. As these cases generally have normal serum taurine levels, they are once more classified as “idiopathic dilated cardiomyopathy”. In addition to humans and cats, primary (idiopathic) dilated cardiomyopathy has also been reported in turkeys (O'Brien *et al.*, 1993), cattle (Van Vleet and Ferrans, 1986; Weekes *et al.*, 1999), ferrets (Lipman *et al.*, 1987) and dogs.

#### 1.1.5 Canine DCM

Most cases of DCM in dogs are assumed to be idiopathic. There are instances, however, where a specific causal factor has been identified. Myocardial disease in the dog has been reported secondary to doxorubicin toxicity (Mauldin *et al.*, 1992), parvovirus infection (Atwell and Kelly, 1980), and infection with



*Borrelia burgdorferi* (Levy and Duray, 1988). Spontaneous supraventricular tachycardia can cause myocardial failure, which is reversible following correction of the tachyarrhythmia (Wright *et al.*, 1999). There is a case report of suspected postpartum cardiomyopathy in a Doberman bitch (Sandusky and Cho, 1984), although the high prevalence of DCM in this breed throws doubt on the significance of her post-parturient state.

There are other cases where there may be an underlying metabolic abnormality resulting in DCM. Although L-carnitine is not an essential nutrient in dogs, L-carnitine deficiency was reported in a group of related Boxer dogs with DCM (Keene *et al.*, 1991). Based on extrapolation from small numbers of dogs found to have low myocardial carnitine levels, it has been suggested that as many as 40% of dogs with DCM may be L-carnitine deficient (Keene, 1991). While this appears highly improbable, there are few data available reporting myocardial carnitine levels in large samples of dogs with DCM, owing to the impracticality of performing endomyocardial biopsies for collecting tissue samples. However, the results of supplementing L-carnitine in dogs with DCM have been variable, and current recommendations are limited to considering supplementation in boxers with DCM only (Costa and Labuc, 1994; Keene *et al.*, 1989; Pion *et al.*, 1998). There have been a number of reports of low plasma taurine levels in asymptomatic dogs and dogs with naturally-occurring dilated cardiomyopathy, with American cocker spaniels and golden retrievers at possible increased risk (Kittleson *et al.*, 1997; Kramer *et al.*, 1995). Supplementation with taurine alone has not been found to be consistently helpful in ameliorating signs of DCM, and one study suggested supplementation with a combination of taurine and L-carnitine was necessary

(Gavaghan and Kittleson, 1997; Kittleson *et al.*, 1997). In the group of dogs examined by Kittleson and others (1997), the study was not a cross-over design, and the study was unblinded after several months. Some of the placebo dogs were also censored because of an unexplained increase in plasma taurine levels. While this study supports a role for taurine and L-carnitine supplementation in American cocker spaniels with DCM and low plasma taurine levels, the case for taurine deficiency as a cause of DCM in dogs remains unproven.

The high prevalence of DCM in certain breeds of dogs argues strongly for a familial tendency in many cases of canine DCM, and there is much active work focussing on the search for a genetic cause (Dukes McEwan, 1999; Meurs, 1998; Meurs *et al.*, 1999; Schatzberg *et al.*, 1999).

#### 1.1.6 Pathological findings in canine DCM

As in man, canine DCM is defined as a condition of unknown aetiology which results in decreased cardiac contractility, chamber dilation with extensive left ventricular remodelling and progressive congestive cardiac failure (Fox, 1988; Thomas, 1987).

Gross pathological findings in affected dogs include a flaccid heart with dilation of all four cardiac chambers (although sometimes the right side is minimally affected), with increased circumferences of the atrioventricular valves and atrophied papillary muscles (Darke and Else, 1984). The ventricular walls may be normal or reduced in thickness despite an increased heart: bodyweight ratio (Fox, 1988; Van Vleet *et al.*, 1981). Concurrent endocardiosis of the atrioventricular valves is sometimes recorded (Darke and Else, 1984; Whitney, 1974). Areas of pale, fibrotic

endocardium have been found inconsistently (Van Vleet *et al.*, 1981), although there are recent reports of characteristic changes in cardiomyopathic Dobermanns that may be visible grossly as pale linear areas in longitudinal sections of the LV free wall (Everett *et al.*, 1999).

The most commonly encountered histopathological changes appear to be scattered focal areas of fibrosis and myocardial necrosis (Sandusky *et al.*, 1984; Van Vleet *et al.*, 1981), particularly in the areas of the papillary muscles and subendocardium. Recently, there have been reports of attenuated wavy fibres being a consistent finding in dogs with DCM (Tidholm *et al.*, 1998). Everett and others (1999) reported a striking distribution of lesions in Dobermann DCM, with the LV free wall being almost exclusively affected, and characterised by myofibre degeneration and atrophy, and replacement of myocardium by dense bundles of collagen and clusters of adipocytes. Medial hyperplasia of intra-mural coronary arteries also appears to be a common finding, although its significance is unclear as similar changes may be seen in old, unaffected dogs (Darke and Else, 1984; Van Vleet *et al.*, 1981).

Inflammatory infiltrates and fibrofatty replacement have been reported in Boxer cardiomyopathy (Harpster, 1983; Wotton, 1998b). Boxer cardiomyopathy is currently receiving attention in recognition of the similarities with arrhythmogenic right ventricular dysplasia in humans (Fontaine *et al.*, 1995; Kraus *et al.*, 1999; Thiene *et al.*, 1988; Wotton, 1999). Boxers with cardiomyopathy frequently exhibit ventricular arrhythmias with a left bundle branch block morphology, and have a high incidence of sudden death.

Ultrastructural changes in canine DCM are generally similar to those reported in man, ie. Z-band abnormalities, myocytolysis, lipid vacuoles and mitochondria alterations (Bishop, 1986; Sandusky *et al.*, 1984; Wynne and Braunwald, 1997).

None of these gross pathological, histological or ultrastructural features are generally considered pathognomonic for idiopathic DCM, as similar changes can be found in myocardial failure secondary to other cardiac diseases (Bishop and Cole, 1969; Richardson *et al.*, 1996; Wynne and Braunwald, 1997), although Tidholm's group argue that attenuated wavy fibres may prove to be a specific marker (1998). Currently, as in man, the diagnosis is generally one of exclusion in individuals with myocardial failure and suggestive historical and physical findings, although coronary artery disease is seldom specifically excluded as a cause. Despite a recent report suggesting ischaemic heart disease may be more common in dogs than previously thought (Falk and Jönsson, 2000), coronary artery disease is not prevalent in this species (Luginbuhl and Detweiler, 1965; Pensinger, 1968).

#### 1.1.7 Clinical features of canine DCM

Idiopathic dilated cardiomyopathy was first reported as a specific canine condition in 1970, by Ettinger and Suter, and Ettinger, Bolton and Lord (Ettinger *et al.*, 1970; Ettinger and Suter, 1970). Prior to that, naturally-occurring myocardial disease had been reported by a number of authors (Detweiler *et al.*, 1968; Taylor and Sittnikow, 1968), but these reports included myocarditis and other conditions which can lead to secondary myocardial damage. Ettinger and Suter (1970) described a condition chiefly affecting large breeds of dog (including Dobermanns), that was

characterised by ventricular and atrial dilation, and progressive congestive heart failure. Although mitral and tricuspid insufficiency were often present, the atrioventricular valves were generally normal, or nearly normal. The authors noted that atrial fibrillation was the predominant rhythm, and that affected dogs rarely survived for longer than six to twelve months after the onset of clinical signs. Around the same time in the UK, mitral incompetence was described in a series of young Great Danes and German shepherd dogs, associated with a normal mitral valve but left atrial and ventricular dilation on post mortem examination (Dear, 1971). It was concluded that the cause of the mitral insufficiency was dilation of the mitral annulus, although Dear did not identify the underlying aetiology.

These characteristics described by Ettinger and Suter have remained essentially unaltered in subsequent descriptions (Calvert, 1992; Darke, 1985; Fox, 1988; Keene, 1989; Thomas, 1987; Ware, 1992; Wood, 1983). The overall incidence has not been reported (Buchanan, 1992; Fioretti and Delli Carri, 1988), although most authors acknowledge that DCM is relatively common. In most reports, males have been described as being more commonly affected than females. Middle-aged dogs are the commonly reported age group, and the risk increases with age (Sisson *et al.*, 1999) although young Portuguese water dogs have been reported to be affected (Sleeper *et al.*, 1998).

All reports agree that DCM overwhelmingly affects pure-bred dogs, and that certain breeds are particularly affected. Giant breeds are represented in most reports (Great Danes, St Bernards, Irish Wolfhounds), as are Dobermanns. DCM appears to be uncommon in dogs of less than 15kg (Keene, 1989). It is recognised that dilated cardiomyopathy in certain breeds may be consistently associated with certain clinical

characteristics; as already mentioned, Boxer cardiomyopathy is frequently associated with a high prevalence of ventricular arrhythmias compared with DCM in giant breeds (Harpster, 1983). Dobermanns (called Doberman pinschers in the USA) and cocker spaniels with DCM show consistent variations from giant breed dogs with DCM (Calvert, 1986; Gooding *et al.*, 1982).

### 1.1.8 History and physical findings

Despite documentation of the gradual development of left ventricular dysfunction prior to the development of congestive heart failure, the occult phase is frequently missed, and the presenting history in dogs with DCM is typically fairly acute, relating to the onset of congestive cardiac failure. Typical signs include coughing, dyspnoea, exercise intolerance or abdominal distension. Some dogs may also be lethargic, inappetent and may lose weight. Physical examination may reveal signs of low cardiac output and peripheral vasoconstriction (pale mucous membranes with prolonged capillary refill time, tachycardia and weak pulse, hypothermia and cold extremities). Signs of left heart failure include tachypnoea and pulmonary crackles on inspiration. Right heart failure may be evidenced by jugular venous distension, muffled heart sounds and a pleural fluid line on thoracic percussion, and ascites with hepatic enlargement. Subcutaneous oedema is uncommon. Arrhythmias are generally present, and an S3 gallop sound is often heard. Systolic murmurs of mitral and tricuspid insufficiency may be present, but are generally of low intensity.

### 1.1.9 Ancillary diagnostic tests

Electrocardiographic changes suggestive of left ventricular and left atrial enlargement are frequently encountered, and left bundle branch block may also be common in some breeds (eg. Boxers). Atrial fibrillation is by far the most frequently encountered arrhythmia (particularly in giant breeds). Ventricular arrhythmias are also common, and supraventricular tachycardias are occasionally seen (Brownlie, 1991; Brownlie and Nott, 1991). Radiography usually demonstrates marked generalised cardiomegaly, with pulmonary venous distension and hilar interstitial infiltrates caused by pulmonary oedema. Associated echocardiographic findings will be described in detail in the relevant chapters. Clinicopathological abnormalities may be observed secondary to the neurohormonal, renal and hepatic effects of heart failure. Hyponatraemia, azotaemia and elevated liver enzymes are frequently noted (O'Brien *et al.*, 1993). Increased catecholamines levels have also been reported (O'Brien *et al.*, 1993; Ware *et al.*, 1990), although they have not been related to survival times or prognosis, despite the good correlations found in human myocardial disease.

### 1.1.10 Treatment of canine DCM

Treatment of dogs with DCM was for many years based on diuresis and use of cardiac glycosides (Ettinger and Suter, 1970b; Kittleson *et al.*, 1985). More recently, the effects of angiotensin converting enzyme inhibitors such as enalapril have proved encouraging (Ettinger *et al.*, 1998; The COVE Study Group, 1995; The IMPROVE Study Group, 1995). With the exception of digoxin, orally active positive inotropes have not been commercially available for use in dogs until

recently. Positive inotropic support traditionally had to be given by intravenous infusion. The most widely used is dobutamine, but there have been several reports of the beneficial effects of milrinone (Calvert, 1991c; Keister *et al.*, 1990; Kittleson, 1991; Kittleson *et al.*, 1987). Pimobendan (a benzimidazole derivative classified as an inodilator) has recently become available for use in dogs in the UK. Its use was shown to increase survival time in Dobermanns with DCM in a small double-blinded placebo-controlled study (Luis Fuentes *et al.*, 1998). The dogs in this report form part of the group described in this thesis.

Beta-adrenergic antagonists have recently proved encouraging as chronic therapy for human DCM (Anonymous, 1999a; Anonymous, 1999b), but their use in canine DCM has not yet been widely adopted, despite encouraging experimental evidence (Sabbah *et al.*, 1994; Tsutsui *et al.*, 1994).

Antiarrhythmic drugs have been advocated for dogs with DCM and severe ventricular arrhythmias, although there have been few controlled studies of their efficacy. One study of tocainide in Dobermanns with DCM showed a reduction in the frequency of ventricular premature complexes and ventricular tachycardia in 90% of treated dogs on follow-up Holter recordings (Calvert *et al.*, 1996c). Sotalol is being increasingly used as an antiarrhythmic in dogs with DCM, with anecdotal reports of improved survival times (Meurs and Brown, 1998).

Survival times of affected dogs vary from less than 8 weeks after the development of congestive failure for Dobermanns (Calvert *et al.*, 1997), to several years for giant breeds and cocker spaniels (Wotton, 1992; Wotton, 1998a).



Whilst sharing many common features, there are specific variations from the usual clinical characteristics of DCM in Dobermanns and cocker spaniels with DCM. These are reviewed in chapter two.

## 1.2 M-MODE ECHOCARDIOGRAPHY

M-mode echocardiography produces a graphic display of the structures of the heart reflected by an “ice-pick” shaped beam of ultrasound plotted against time. With frame update rates which far exceed those of other echocardiographic modalities (1000-5000 pulses per second), M-mode echocardiography maintains a place in clinical cardiology as a result of its superior temporal resolution.

### 1.2.1 History of M-mode echocardiography

M-mode echocardiography was the first form of echocardiography to be used in clinical medicine. In 1956, Edler and Hertz adapted an echograph designed for metal flaw detection for use in human patients, and subsequently applied this “time-motion” or “motion-mode” ultrasound technique to mitral valve disease. Over the following ten years, other applications for “M-mode echocardiography” were described; including detection of left atrial myxoma (Effert and Domanig, 1959) and pericardial effusions (Feigenbaum *et al.*, 1965).

In the late 1960s, M-mode echocardiography found a new application in the quantitative measurement of ventricular dimensions (Feigenbaum *et al.*, 1968; Popp *et al.*, 1969). Prior to this, the only technique for measuring ventricular wall thickness had been angiography. M-mode echocardiography continued to develop

over the subsequent ten years. As well as enabling the objective measurement of cardiac structures, M-mode echocardiography ushered in a new era of non-invasive evaluation of left ventricular function. Previously, invasive techniques such as angiography had been necessary to evaluate systolic function.

### 1.2.2 M-mode measurement and reproducibility

A number of studies have examined variability in measurements made by M-mode echocardiography (Bett and Dryburgh, 1981; Felner *et al.*, 1980; Friedman *et al.*, 1982; Grandits *et al.*, 1994; Pietro *et al.*, 1981; Vignola *et al.*, 1977). Left ventricular diameter measurements appear to be more reproducible than septal and free wall thickness (Grandits *et al.*, 1994; Pietro *et al.*, 1981). Heart rate variability was an important source of variation, and was considered to be responsible for some of the observed day-to-day variation (Bett and Dryburgh, 1981; Felner *et al.*, 1980). In a study of sources of variability of echocardiographic measurements by Felner and others (1980), one of the major sources of variability was related to the reproducibility of intra-observer and inter-observer measurements, rather than the sonographer or day-to-day variation of the subjects. In some instances, the within-observer variance was as great or greater than between-observer variation, leading to the suggestion that every echo should be interpreted at least twice, and preferably by two different observers. Another study found more reliable agreement between successive readings when made by cardiologists rather than sonographers (Grandits *et al.*, 1994).

In 1978 the American Society of Echocardiography (ASE) established guidelines for the measurement of M-mode echocardiograms (Sahn *et al.*, 1978).

These recommendations were based on the results of 400 questionnaires distributed to members with an interest in M-mode echocardiography. The questionnaires consisted of five sample echocardiograms, and the respondents were asked to measure a series of dimensions, listing the criteria they used to define the exact markers for each measurement. In fact, the respondent rate was only 19%, and for most measurements there was no clear consensus. The percentage uncertainty (defined as the 95<sup>th</sup> percentile confidence limit divided by the mean and multiplied by 100) was calculated for each variable, and used as the basis for identifying the most reproducible criteria.

It was recommended that end-diastolic measurements be made at the onset of the QRS complex on the electrocardiogram (despite the fact that this precedes true end-diastole) because most measurements timed at this point had a lower percentage uncertainty than those made at the peak of the R wave. For left ventricular dimensions it was recommended that end-systolic measurements were made at the timing of peak posterior septal motion, as this corresponded with true end-systole more closely than peak anterior motion of the posterior wall, end of the electrocardiographic T wave, or minimal left ventricular diameter. It was further recommended that measurements were made using the “leading edge” methodology, both when measuring the left ventricular dimensions, and the aorta and left atrium. It was suggested that the aorta be measured at end-diastole, and the left atrium at end-systole (although the exact markers to be used for the timing of end-systole with this measurement were not specified). The left ventricular dimensions were to be obtained at the chordal level, although for children and infants it was suggested that the mitral valve level was more appropriate. Additional recommendations included

use of a lead II electrocardiogram (ECG), and averaging three or four cardiac cycles.

However, inherent limitations with M-mode echocardiography were recognised. One major difficulty was associated with the problems in obtaining consistent alignment. The original technique employed a single beam of ultrasound, which was aimed at the chest wall without any two-dimensional imaging for guidance. When two-dimensional echocardiography was introduced in the late 1970s, the difficulties of obtaining a true plane through the ventricular meridian with unguided M-mode became apparent (Hoenecke *et al.*, 1982). It was shown to be impossible to determine the accuracy of alignment solely from the apparent quality of the M-mode tracing. It was also recognised that the heart moved in three planes in systole relative to diastole, so that it was nearly impossible to view identical regions of the heart throughout the cardiac cycle (Moses and Ross, Jr., 1987). Some of these problems (though not all) were resolved with the advent of 2D-guided M-mode, which permitted a simultaneous on-screen assessment of the alignment of the M-mode cursor with the left ventricle. Nevertheless, Pietro and co-workers (1981) did not find any improvement in reproducibility using 2D-guided M-mode compared with unguided M-mode with experienced sonographers and cardiologists, using well-defined measurement criteria.

Although the main aim behind the ASE recommendations had been to establish criteria which would result in the most reproducible measurements, Crawford and others (1980) criticised the guidelines, suggesting an alternative technique that resulted in more accurate measurements without any loss of repeatability. It should be noted that the 'gold standard' used in this study was angiographically calculated volumes, with M-mode volumes being calculated by

cubing the minor axis dimensions. Crawford's technique involved making end-diastolic measurements at the peak of the R-wave on the ECG, and extrapolating systolic dimensions from the peak excursion of the free wall. In fact, a study by Friedman and others (1982) comparing M-mode values with measurements using implanted sonomicrometer crystals in a dog model (i.e., direct validation), showed that both ASE conventions and Crawford's technique correlated well with the invasive method, but using the largest and smallest LV diameters for end-diastolic and end-systolic dimensions did not. It should be emphasised that good correlation does not necessarily indicate good agreement, as correlation does not provide information on bias.

Picard has criticised the recommendations more recently for different reasons (Picard, 1994). He pointed out that a current problem with using the ASE recommendations (measured as leading edge to leading edge) means that measurements will necessarily differ from those derived from 2DE images (measured as inner edge to inner edge based on ASE recommendations (Schiller *et al.*, 1989)).

Other M-mode guidelines (O'Rourke *et al.*, 1984; Roelandt and Gibson, 1980) do not really differ in their recommendations from the ASE guidelines, apart from suggesting the timing of end-systole is based on the aortic component of the 2<sup>nd</sup> heart sound by phonocardiography.

### 1.2.3 M-mode echocardiography in the assessment of cardiac dimensions

M-mode echocardiography was rapidly recognised as a non-invasive tool for the assessment of cardiac dimensions in human subjects, along with the need for standard reference intervals (Henry *et al.*, 1980b). Echocardiographic values were typically plotted against age with 95% prediction intervals, both for adults and children (Gardin *et al.*, 1979; Gutgesell *et al.*, 1977). Gutgesell and others (1977) also noted a strong correlation with the logarithm of bodyweight in children ( $r = 0.95$ ). Subsequent studies have acknowledged the influence of height, body surface area and gender (Huwez *et al.*, 1994; Lauer *et al.*, 1995; Vasan *et al.*, 1997). The study by Vasan and co-workers (1997) was based on data from the Framingham study, and provided reference intervals based on the 95<sup>th</sup>, 98<sup>th</sup> and 99<sup>th</sup> percentile intervals according to gender in 4957 patients, thereby allowing classification of patients according to their degree of deviation from the norm.

Vasan and others (2000) recently reviewed the major reports of M-mode and 2D reference intervals in the literature, and called for standardisation of reference limits according to clinically useful partitions such as gender, height, and race, etc.

### 1.2.4 M-mode echocardiography and LV volume measurement

Some of the problems of deriving left ventricular volumes from M-mode measurements included difficulties in obtaining a true minor axis (Linhart *et al.*, 1975), and measuring different parts of the ventricle in systole and diastole (Bhatt *et al.*, 1978). The formula used for calculation of volume also affected reliability: most

formulae relied on a variation of the cube formula, where left ventricular volume is equal to the cube of the minor axis dimension (Feigenbaum *et al.*, 1972; Fortuin *et al.*, 1972; Murray *et al.*, 1972; Pombo *et al.*, 1971). However, it was shown that the reliability in enlarged ventricles was much reduced, as the ratio of minor dimension to major dimension changed with dilation of the ventricle. Teichholz and co-workers (1976) examined left ventricular angiograms in 100 human patients (both normal and with a variety of cardiac diseases) with respect to the ratio of the long axis to minor axis dimensions. The relationship was curvilinear, and a corrected cube formula was derived which correlated more closely with angiographically derived volumes over a range of ventricular geometries. Nevertheless, even this formula was only held valid for symmetrically contracting ventricles. Furthermore, any error in measurement of the minor axis would be compounded by a volume estimation that was based on cubing the incorrectly measured dimension.

### 1.2.5 M-mode echocardiographic assessment of ventricular function

M-mode measurements used for functional assessment have included left ventricular fractional shortening (Gutgesell *et al.*, 1977), percentage wall thickening (Sasayama *et al.*, 1976) mitral valve E-point to septal separation (Child *et al.*, 1981), and estimation of left ventricular ejection fraction (Teichholz *et al.*, 1976).

#### *1.2.5.1 LV fractional shortening*

Left ventricular fractional shortening was one of the earliest echocardiographic measurements to be used in the assessment of ventricular function

(Fortuin *et al.*, 1972; McDonald *et al.*, 1972). It proved to be a simple measurement to make, and was found to be independent of heart rate, as well as age-independent in children and adults (Gardin *et al.*, 1979; Gerstenblith *et al.*, 1977; Gutgesell *et al.*, 1977). Nevertheless, the demand for an echocardiographic equivalent of the angiography-derived ejection fraction led to the derivation of a number of formulae using M-mode measurements, despite the disadvantage of only measuring shortening in one dimension.

#### *1.2.5.2 LV wall thickening*

Wall thickening was another measurement that was also used as an index of global ventricular function, and was free of any errors associated with squaring or cubing the measured value. Sasayama and others (1976) measured wall thickening directly in dogs using ultrasonic crystals. A variety of interventions were used to alter ventricular function, including atrial pacing, administration of phenylephrine, isoprenaline, and propranolol, and coronary occlusion. They concluded that percentage wall thickening correlated well with changes in conventional echocardiographic indices such as fractional shortening and velocity of circumferential fibre shortening, and that wall thickening could be used as an index of global ventricular function in the absence of regional wall motion abnormalities. Studies in human patients showed reduced percentage wall thickening in coronary artery disease and dilated cardiomyopathy, with normal wall thickening persisting in patients with atrial septal defects who had abnormal wall motion (Corya *et al.*, 1977).



#### *1.2.5.3 Mitral E-point to septal separation*

Mitral-septal separation (EPSS) was another simple index that could be obtained from an M-mode recording at the mitral valve level. The maximal separation between the anterior mitral valve leaflet at the E-point excursion in early diastole and the septal wall was shown to correlate well with angiographic ejection fraction (Massie *et al.*, 1977). Left ventricular size did not appear to influence EPSS in a study by Child and others (1981), and appeared to be a reliable indicator of LV systolic function in children (Engle *et al.*, 1983).

#### *1.2.5.4 Systolic time intervals*

Systolic time intervals were used for the noninvasive assessment of ventricular performance before their application to M-mode echocardiography. Originally derived from the electrocardiogram, phonocardiogram and carotid pulse tracings, the pre-ejection period (PEP) and left ventricular ejection time (LVET) were shown to correlate reasonably well with invasive indices of left ventricular contractility (Weissler *et al.*, 1968). A number of studies in human patients showed that PEP was independent of heart rate, but increased with a fall in myocardial performance (decreased contractility, decreased preload or increased afterload) and decreased with an improvement in ventricular performance. LVET was inversely proportional to heart rate, and decreased with both positive and negative inotropes, and decreased with a fall in preload. The ratio PEP/LVET correspondingly increased with a deterioration in ventricular performance, and decreased with an improvement in ventricular performance (Ahmed *et al.*, 1972; Hassan and Turner, 1983; Lewis *et al.*, 1977).

It was subsequently demonstrated that systolic time intervals could be accurately calculated from aortic valve M-mode recordings with a concurrent ECG, adding a further dimension to the echocardiographic assessment of ventricular function (although these indices were still load-dependent) (Gutgesell *et al.*, 1977; Spodick *et al.*, 1984). Mean velocity of circumferential fibre shortening (Vcf) was an additional index that divided the left ventricular fractional shortening by the LVET (Fortuin *et al.*, 1972). Although Vcf did not alter with age in adults (Gerstenblith *et al.*, 1977), it was found to be higher in young children with high heart rates (Gutgesell *et al.*, 1977).

#### 1.2.6 Assessment of left atrial size

Left atrial size was first measured by M-mode echocardiography, and the ratio of left atrial diameter to aortic diameter was rapidly adopted as a means of standardising atrial dimensions in individuals of differing size. For this ratio, the left atrial diameter is measured in an anterior-posterior dimension, from a right parasternal view that includes the aorta and left atrium. As with other M-mode measurements, a leading edge technique was recommended by the American Society of Echocardiography (Sahn *et al.*, 1978). It was also recommended that atrial diameter was measured at end-systole, when atrial volume is at its maximum (Sahn *et al.*, 1978). In contrast, end-diastole is used for aortic measurements.

## 1.2.7 M-mode echocardiography in dogs

### 1.2.7.1 Normal dogs

Normal M-mode values in dogs were reported in a number of papers (Bonagura *et al.*, 1985; Boon *et al.*, 1983; Lombard, 1984b; Mashiro *et al.*, 1976). It should be noted that most of these reported values were from unguided M-mode studies rather than 2D-guided M-mode. Caution should probably be exercised when relating these values to properly aligned 2D-guided M-mode results.

As in human cardiology, the introduction of 2D echocardiography led to an expansion of diagnostic capabilities in canine cardiac disease, but the value of M-mode continued to be recognised as the most practical means of measuring cardiac chamber dimensions. Some of the early reports correlated chamber dimensions with body weight, with the publication of confidence intervals for regression lines of M-mode values against bodyweight (Bonagura *et al.*, 1985; Lombard, 1984b) and body surface area (Boon *et al.*, 1983).

One specific veterinary problem with normal M-mode reference intervals was the confounding influence of breed (Morrison *et al.*, 1992). The heterogeneity of cardiac size and shape amongst different dog breeds prompted studies of M-mode values for specific breeds of dog. Beagles had been one of the earliest breeds reported (Dennis *et al.*, 1978), and new studies in this breed continue to be reported (Crippa *et al.*, 1992; Hanton *et al.*, 1998). Values in Dobermanns and English cocker spaniels were also reported relatively early, partly because of the incidence of myocardial disease in these breeds (Calvert *et al.*, 1982; Gooding *et al.*, 1986a). Other specific breeds with normal values reported include golden retrievers, miniature poodles, Afghan hounds, and Pembroke Welsh corgis (Morrison *et al.*,

1992), greyhounds (Page *et al.*, 1993; Snyder *et al.*, 1995), boxers (Herrtage, 1994), Spanish Mastiffs (Bayon *et al.*, 1994), Irish Wolfhounds, Great Danes, and Newfoundlands (Dukes McEwan, 1998b; Koch *et al.*, 1996). Further reports of normal values in Dobermanns include the study by Sottiaux and Amberger (1997), and the study by Minors and O'Grady (1998). Smucker and co-workers found subnormal systolic function in asymptomatic Dobermanns compared with mongrels, using both %PWth and angiographic ejection fractions (Smucker *et al.*, 1990).

The numbers of subjects reported in most of these studies are relatively small, compounding the difficulties in making assessments in individual cases. The problems are amplified further for mixed-breed dogs, or for pure-bred dogs of a breed for which no reference intervals exist.

As in children, many derived measurements appear to be independent of body size; including left ventricular FS%, EPSS, %PWth, Vcf, and LA:Ao (Boon *et al.*, 1983; Kirberger, 1991).

#### *1.2.7.2 Dogs with cardiac disease*

M-mode echocardiography had been applied in canine models of cardiac disease as early as 1965 (Feigenbaum *et al.*, 1965). M-mode echocardiography was subsequently applied to a variety of acquired cardiac conditions, including mitral regurgitation (Kittleson *et al.*, 1984; Lombard and Spencer, 1985; Pipers *et al.*, 1981) pericardial effusion (Bonagura and Pipers, 1981) bacterial endocarditis (Lombard and Buergelt, 1983) and dilated cardiomyopathy (Bonagura and Herring, 1985).

In the absence of breed-specific reference intervals, identification of chamber enlargement using M-mode echocardiography has been based on reference ranges based on bodyweight. Lombard and Spencer (1985) compared the agreement between radiography and M-mode echocardiography in identifying ventricular enlargement in dogs with mitral regurgitation. They divided the measured M-mode dimensions by the expected echocardiographic dimensions normalised for bodyweight to derive an echocardiographic enlargement ratio, and found unreliable agreement with mild chamber enlargement (Lombard, 1984b; Lombard and Spencer, 1985). While radiography cannot be considered to be a “gold standard” for assessment of left ventricular enlargement, this study illustrated some of the practical difficulties encountered in making decisions about chamber enlargement in individual cases.

Although FS% has been the most widely used index of left ventricular function, other measurements have also been used. Atkins and co-workers (1992) compared cardiac index (cardiac output indexed to body surface area) measured by M-mode echocardiography with cardiac index measured by thermodilution in dogs. They compared four different formulae for calculation of cardiac output, using normal dogs under different levels of inotropic stimulation, experimentally induced heartworm disease, and dogs with DCM. Even in normal dogs under baseline conditions, the correlation was modest ( $r^2 = 0.54-0.68$ ), and was worse under inotropic stimulation, with no significant correlation in the presence of cardiac disease (Atkins *et al.*, 1992). Vcf has also been used (Atkins and Snyder, 1992; Calvert and Brown, 1986).

Endsystolic volume index (ESVI) has been used to identify dogs with myocardial failure secondary to mitral regurgitation based on a human classification system devised by Borow (Borow *et al.*, 1980; Kittleson *et al.*, 1984; Kittleson *et al.*, 1985; Ware *et al.*, 1990). There are several difficulties with using such a system. There are major limitations with calculating LV volumes from minor axis dimensions, especially in dilated ventricles. The formula used in Kittleson's studies was based on the Teichholz formula, which includes a constant derived empirically using human patients to circumvent the problems associated with dilated ventricles (Teichholz *et al.*, 1976). Kittleson acknowledged in his study that the accuracy of this equation had not been evaluated in dogs (Kittleson *et al.*, 1984), but did not acknowledge the shortcomings of using cut-off points for ventricular volumes based on a human classification. Indexing canine echocardiographic dimensions to body surface area does not appear to cancel out the effects of bodyweight across different breeds. When LV diastolic dimensions are indexed to body surface area and plotted against bodyweight, smaller breeds of dog have a relatively higher LV end-diastolic diameter index than large breeds (Bonagura and Luis Fuentes, 1999). Nevertheless, Kittleson's study is often quoted as though these cut-off points have been validated in dogs (Boon, 1998a).

It has been suggested that EPSS is one of the most sensitive and specific indicators of early dilated cardiomyopathy in Dobermanns (Calvert and Brown, 1986), and is an additional useful indicator of myocardial failure (Bonagura and Herring, 1985). Percentage free wall thickening has been used far less than FS%, even though the measurements required for deriving %PWth are often available (Lombard, 1984a). Kittleson, Knowlen and Johnson (1987) measured percentage

wall thickening in a canine model of myocardial infarction, and found that wall thickening did not improve post-infarction even when wall motion returned. They indicated that other factors (such as wall stiffness) could allow abnormal regions of the posterior wall to move passively, contributing to increased wall motion; whereas wall thickening was more directly related to contractile function.

#### *1.2.7.3 M-mode assessment of left atrial size in dogs*

M-mode echocardiography has been used to measure left atrial size in veterinary medicine, almost to the exclusion of every other echocardiographic technique. Although the convention for recording and measuring M-Mode images at the left atrial level suggested by the ASE has been followed by virtually all veterinary echocardiographers, the plane used is not the same as in human patients. In human subjects (and in cats) it is very straightforward to obtain a right parasternal long axis view that includes the aorta in long axis, and part of the left atrium with the mitral annulus included. In dogs, it is very difficult to obtain a right parasternal long axis view that contains more than part of the left atrium in the same plane as the long axis aorta. By the same token, the right parasternal short axis view in humans does not include the left auricular appendage with the body of the left atrium. In dogs, it is almost impossible to avoid the left auricular appendage if the M-mode cursor crosses the aortic valves. This means that left atrial dimensions measured by M-mode in dogs are even less reliable indicators of left atrial enlargement than in people.

The M-mode ratio of LA:Ao became a standard way of assessing left atrial enlargement (Calvert and Brown, 1986; Lombard and Spencer, 1985; Wingfield *et*

*al.*, 1982). Many early canine M-mode studies included left atrial and aortic dimensions, and their ratio was consistently found to be independent of bodyweight or body surface area. Lombard and Spencer (1985) attempted to use a ratio of measured LA size to predict LA size (based on bodyweight) in an effort to identify LA dilation in dogs with mitral regurgitation. They found improved agreement with radiographic assessment of LA size when they used the LA:Ao ratio in the same dogs (Lombard and Spencer, 1985).

Values for normal canine left atrial : aortic ratios on M-mode have ranged from 0.89 for normal Dobermanns (Calvert and Brown, 1986) to 1.2 for normal poodles (Morrison *et al.*, 1992), probably depending on the ease of alignment with the body of the left atrium (the left auricular appendage is always narrower than the body of the left atrium, and it appears to be particularly difficult to avoid in deep-chested dogs).

M-mode findings in dogs with DCM are discussed in more detail in chapter three.

### 1.3 QUANTITATIVE TWO-DIMENSIONAL ECHOCARDIOGRAPHY

The advent of two-dimensional echocardiography (2DE) provided much welcomed improvements in diagnostic imaging, with its tomographic slices of the heart. The transducer emitted an ultrasound beam that moved in an arc, with reconstruction of the information from the received signals as a video image in a sector shape. Early machines moved the transducer piezoelectric crystal mechanically, with phased array transducers triggering an array of individual crystals



electronically. Annular array probes use a combination of mechanical and phased array technology.

### 1.3.1 2DE and the assessment of cardiac volumes

Although the major application of 2DE has probably been in the imaging of cardiac lesions, 2DE has established a firm place in quantitative echocardiography as a widely accepted technique for calculation of left ventricular volumes. The requirement for a noninvasive echocardiographic equivalent to the angiography-derived ejection fraction led to the adaptation of M-mode dimensions to calculate left ventricular volumes (Feigenbaum *et al.*, 1972; Fortuin *et al.*, 1972; Murray *et al.*, 1972; Pombo *et al.*, 1971; Teichholz *et al.*, 1976).

#### *1.3.1.1 Validation of 2DE-derived LV volumes in isolated hearts*

Early studies comparing M-mode echocardiography, 2DE and direct volume estimation in canine models readily established the superiority of 2DE volume calculations over those derived by M-mode, or based on a single minor axis dimension (Schapira *et al.*, 1981; Wyatt *et al.*, 1980a; Wyatt *et al.*, 1980b). Some 2DE volume formulae were based on those used in angiocardigraphic volumetric techniques, employing the same principles of measuring major and minor ventricular dimensions or planimetered areas from single planes or biplane images (Dodge *et al.*, 1966). These include variations on Simpson's rule (serial sections of equal diameter, with the total volume calculated by summing the calculated section volumes) and formulae based on the volume of an ellipsoid. Eaton and others (1979) applied 2DE

to isolated beating canine left ventricles, calculating volume from a volumetric tank connected to a balloon inside the ventricle (Eaton *et al.*, 1979). Simpson's rule was then applied by recording serial short axis sections at 3mm intervals. Correlations with directly measured volume were excellent ( $r = 0.972$ ). In a progression of this work, Weiss and co-workers (1983) repeated the studies, using progressively fewer sections (Weiss *et al.*, 1983). Although the correlation with directly measured volume remained good with even one "slice" ( $r = 0.855$ ), the accuracy was markedly reduced with fewer sections, so that the mean percent error increased from 6.6% with over 15 sections, to 26.6% with a single section. This illustrates one of the problems with relying on correlation coefficients for assessing the relative usefulness of two quantitative techniques.

Wyatt and others (1980) compared direct volume measurement with a number of echocardiographic formulae for quantifying volume in formalin-fixed canine hearts placed in mineral oil. A series of short axis views were obtained for each heart, with a long axis view obtained at  $90^\circ$  to the short axis sections. The formulae examined are displayed in figure 1.1, and included Simpson's rule (formula i), a variety of short-axis area-length formulae based on ellipsoidal geometry (formulae ii – v), a short-axis diameter-cubed formula as used in M-mode calculations (formula vi), and a long axis area-length formula (formula vii). The best correlation with directly measured LV volume was obtained with Simpson's rule ( $r = 0.982$ , mean percent error 9.6%), although all the formulae that used a short-axis area resulted in correlation coefficients of 0.969 or greater.

Of these short axis area formulae, mean % errors ranged from 17.9% for a half-cylinder, half ellipsoid model (figure 1.1, formula iv) up to 31.9% for a

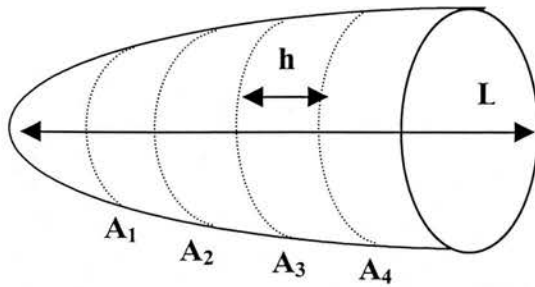
cylindrical model. The poorest correlation was found with the diameter-cubed formula ( $r = 0.837$ ), which had a mean % error of 49.9%. However, Simpson's rule is a difficult formula to use clinically, as it requires a series of parallel short axis views at different levels of the heart. A modification of Simpson's rule uses planimetry of one or two orthogonal long axis views, allowing the calculation of multiple theoretical short axis slices.

Schapira and co-workers (1981) compared 2DE formulae including a modified Simpson's rule with directly measured volumes in excised canine hearts (Schapira *et al.*, 1981). The hearts were scanned in a water bath in the equivalent of right parasternal long axis, short axis and left apical planes, and a biplane modified Simpson's rule was compared with a single plane area-length calculation ( $8A^2/3\pi L$ ) and a biplane area-length formula ( $8A_1A_2/3\pi L$ ), where A represents area, and L represents LV length. Each of the three imaging sections were substituted into the equations, and compared with the directly measured volumes. All of the area-length and Simpson's rule calculations had correlation coefficients greater than 0.90 when compared with true volumes, except for the single-plane area-length calculation using the short axis area alone.

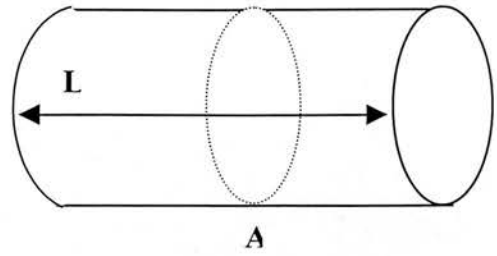
In contrast, the cube formula based on short axis diameter correlated poorly with true volume ( $r = 0.56$ ). The single plane area-length calculations based on long axis and apical planes actually had smaller coefficients of variation than the biplane Simpson's calculations (8.6% and 7.0%, versus 10.0% for the biplane Simpson's formula using the same two views). In none of these studies was there any consistent tendency for 2DE to underestimate or overestimate the true volume, although the

deficiencies of specific formulae would result in deviations from the true volume (e.g. formula (ii) led to overestimation, and formula (vii) led to underestimation).

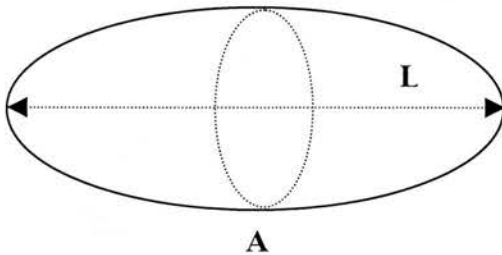
Figure 1.1: LEFT VENTRICULAR VOLUME FORMULAE  
(after Wyatt and others, 1980)



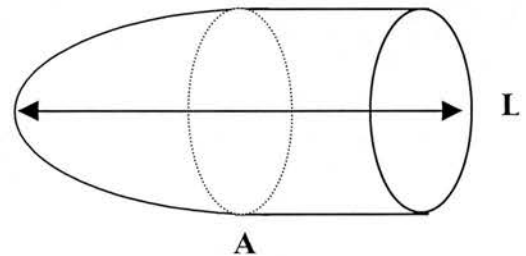
$$\text{Formula (i)} = (A_1 + A_2 + A_3) + \frac{A_4 h}{2} + \frac{\pi h^3}{6}$$



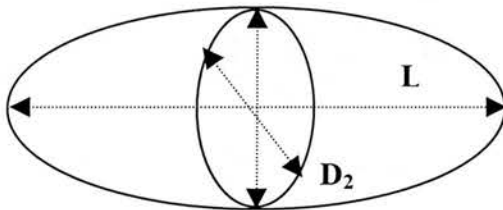
$$\text{Formula (ii)} = AL$$



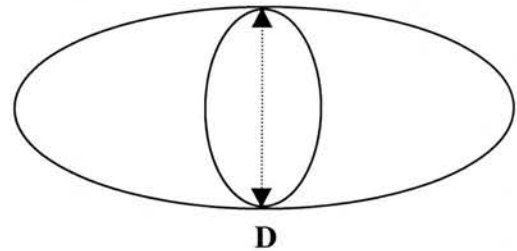
$$\text{Formula (iii)} = \frac{2}{3} AL$$



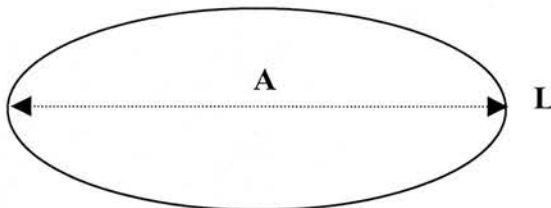
$$\text{Formula (iv)} = \frac{5}{6} AL$$



$$\text{Formula (v)} = \frac{\pi}{6} D_1 D_2 L$$



$$\text{Formula (vi)} = D^3$$



$$\text{Formula (vii)} = 0.85 A^2/L$$

A: area, L: length, D: diameter, h: slice thickness.

### *1.3.1.2 Validation of 2DE-derived LV volumes in canine models*

These studies using excised canine hearts allowed validation of the mathematical formulae used in 2DE volume measurement, as the volumes measured by 2DE were identical to the directly measured volumes. However, there are additional problems associated with in vivo volumetric measurement, not least of which is the difficulty of obtaining an echocardiographic window that permits true long and short axis cross-sections. Collins and others (1982) even ran into difficulties obtaining true long axis views with an open chest canine model (Collins *et al.*, 1982). Furthermore, available in vivo techniques for validating ventricular volume are far from being 'gold standards'. Even direct measurement on excised hearts following closed chest studies is inadequate, because of differences in geometry between the beating heart and excised heart.

More accurate results can be expected with LV mass calculations based on LV wall volume, which are generally based on calculation of the volume within the endocardial borders subtracted from the volume within the epicardial borders, then multiplied by a constant to obtain mass. Left ventricular mass should remain the same throughout systole and diastole, and should be equivalent to the weighed mass of the excised heart, so that studies on LV mass should also provide validation of LV volume estimations (Coleman *et al.*, 1986; Feneley *et al.*, 1988; Schiller *et al.*, 1983; Stack *et al.*, 1987). Wyatt's group (1979) examined the seven formulae listed in figure 1.1 in closed chest, anaesthetised dogs for the purposes of LV mass estimation. Studies were performed with the transducer on the right chest wall, with the animal in right lateral recumbency on a table with a cut-away portion, allowing

the transducer access from beneath the table (as is routine practice in veterinary echocardiography today). They obtained acceptable short axis and long axis images from this window, and confirmed the position of their cross-sections by passing long steel needles through the chest wall in the direction of the ultrasound beam. They found that the entire left ventricle could be imaged in long axis within the 84° sector angle in most dogs under 30kg., and all of their LV length measurements were obtained from this view. Although this paper primarily addressed the comparison of 2DE-derived LV mass with the excised weight, the authors also stated that the lengths measured from this right parasternal long axis view correlated well with the long axis length obtained by cineangiography, being on average only 6.5% shorter with 2DE. They also stated this measurement was highly reproducible, but gave no further supporting figures or data.

#### *1.3.1.3 Comparison of 2DE-derived LV volumes with cineangiography*

Angiography was the standard clinical estimate of LV volume prior to 2DE, and most of the early human 2DE volume studies used cineangiography as a comparison (King *et al.*, 1972; Quinones *et al.*, 1981; Silverman *et al.*, 1980). Gueret and others (1980) compared 2DE volume measurements in closed chest dogs with cineangiography, with additional studies in dogs after left anterior descending (LAD) coronary artery occlusion. Some of these dogs were bigger than in Wyatt's studies (up to 51kg), and it did not prove possible to image the entire left ventricle in long axis from the right side in all of these dogs. An apical view was obtained instead from the left side (though from above the animal this time). Volumes were

calculated either by a Simpson's formula using five short axis areas, or by formula (iv). A consistent underestimation of volume was found with 2DE compared with cineangiography, a finding shared with most other studies using angiography as a comparison (Erbel *et al.*, 1984; Kan *et al.*, 1981; Schiller *et al.*, 1979).

One explanation for this finding is that angiocardiology tends to overestimate true volume, as the contrast-opacified area includes the volume taken up by trabeculae with the luminal volume. Angiography and 2DE have frequently been performed at different times, and the effects of contrast agents might also affect stroke volume.

Erbel and co-workers (1983) answered some of these queries by performing an elegant study (from a theoretical standpoint, if not from the standpoint of the safety of the echocardiography technicians) of simultaneous 2DE and cineventriculography. The transducer was actually included in the cineventriculographic image. They found that in 42/44 subjects, the transducer was anterior and superior to the true anatomic apex when apical images were obtained, largely explaining the 2DE underestimation of dimensions. Volumes were compared on a beat-to-beat basis, and also showed a slight increase in stroke volume with the injection of contrast material, although the effects were minimal compared to the underestimation caused by failing to include the true apex in the 2DE images.

Apical views (four-chamber or two-chamber) (Henry *et al.*, 1980a) are standard for measuring long-axis lengths in human 2DE, recognising the difficulty of obtaining the complete length of the left ventricle from a parasternal long axis view in human beings (Schiller *et al.*, 1989). Even apical views do not afford a consistently good image of the apex, as found in Erbel's study (1983).



Quiñones and others (1981) came up with a novel (if somewhat subjective) solution to the problems in obtaining an apical view that included the apex, by assigning a score for “fractional shortening of the long axis”, depending on the observer’s subjective assessment of apical wall motion. This method allowed volume estimations based on measurements of only the minor axis dimension, but has not been widely used with the advent of readily accessible software packages for planimetry of LV volumes and calculations using a modified Simpson’s rule. In dogs it seems reasonable to circumvent the problems of failing to image the true apex by using a right parasternal view in place of an apical one, as this view appears to yield the maximum long axis length (Feneley *et al.*, 1988; Schiller *et al.*, 1983; Sisson *et al.*, 1989; Stack *et al.*, 1987; Wyatt *et al.*, 1981).

Other techniques compared with 2DE include radionuclide studies (Quinones *et al.*, 1981; Sisson *et al.*, 1989) and more recently, three-dimensional echocardiography (3DE) (Sapin *et al.*, 1996; Siu *et al.*, 1995). As one would expect, 3DE has proved more accurate than 2DE, although image acquisition is more time-consuming (Sapin *et al.*, 1996; Siu *et al.*, 1995). Acoustic quantification is an alternative technique that uses on-line algorithms to identify differences in integrated backscatter between myocardium and blood, allowing automated calculation of LV volumes (Yvorchuk *et al.*, 1994).

#### *1.3.1.4 Clinical use of 2DE for estimation of LV volumes in dogs*

Scant attention has been paid to quantitative 2DE in the veterinary literature. Thomas described most of the standard views in 1984 (Thomas, 1984), when he specifically stated that the apical views did not include the true LV apex in dogs.

Normal values for many 2DE variables were included in a paper by O'Grady and others (1986), which also showed the maximum LV length to be from a right parasternal long axis view. Sisson and others (1989) compared LV volumes calculated by Teichholz's corrected cube formula (Teichholz *et al.*, 1976) with a 2DE short-axis area-length (figure 1.1, formula iv), and radionuclide ventriculography. A couple of recent reviews have mentioned quantitative 2DE measurements, but do not provide any normal reference intervals, or any applications of these measurements (Boon, 1998a; Kienle and Thomas, 1995).

### 1.3.2 2DE assessment of left atrial size

Two-dimensional echocardiography offers obvious advantages over M-mode, allowing measurements of the left atrium to be made in multiple planes. Left apical views allowed measurements of the left atrium in orthogonal views. The right parasternal view often provides poor imaging of the superior border of the left atrium, and this is usually well visualised in apical views. Triulzi and co-workers (1984) compared left atrial measurements made from right parasternal long axis and short axis views, and left apical four-chamber views. Pearlman and others (1990) reported left atrial measurements from a variety of different views in 268 normal human subjects of different ages. Despite these studies, the anteroposterior measurement at the mid-atrial level from a right parasternal long axis view has become the favoured single linear measurement of left atrial size (Oh *et al.*, 1999b; Weyman, 1994).

As with the left ventricle, a more accurate estimate of left atrial size can be obtained by calculation of chamber volume. Similar formulae have been applied to

the left atrium: a modified Simpson's approach can be used with planimetry of orthogonal views such as the four-chamber and two-chamber left apical views. These may be particularly helpful when the left atrial geometry is distorted. Correlation with cine-computed tomography measurements has generally been good; especially when patients with dilated atria are included (Kircher *et al.*, 1991). One study found a better correlation with area/minor length formulae than with Simpson's, possibly due to foreshortening of the major axis of the left atrium in apical views (Vandenberg *et al.*, 1995).

#### *1.3.2.1 2DE assessment of LA size in dogs*

There are few reports of 2DE measurements of left atrial size in dogs. Haendchen and co-workers (1982) measured left atrial size in a closed chest canine coronary occlusion model, making reference to the need to modify the conventional right parasternal long axis view used for human left atrial 2DE measurements (Haendchen *et al.*, 1982). The figure shown in this article is the standard right parasternal long axis view obtained by veterinary cardiologists (Thomas *et al.*, 1993). They reported good repeatability of LA area measured from this view, with interobserver mean percent errors of 4.0% or less. O'Grady and others (1986) reported a number of LA measurements in 14 - 17 dogs of varying weights (O'Grady *et al.*, 1986). The right parasternal view was used to measure a "right-left" dimension (analogous to the anteroposterior dimension) and a "base-apex" dimension (equivalent to the inferior-superior measurement). Left apical views were also used to measure medial-lateral and base-apex dimensions. Larger mean values

were obtained for the right parasternal measurements than for the equivalent apical measurements, suggesting there was some foreshortening in the apical views.

There are even fewer references to 2DE measurements of the canine left atrium in short axis views. Häggström and others have referenced a short axis measurement of left atrial size in a number of studies (Haggstrom *et al.*, 1996; Haggstrom *et al.*, 1994; Haggstrom *et al.*, 1997; Haggstrom *et al.*, 1995). With this measurement, the aortic diameter is measured from the mid-part of the right coronary cusp to the commissures of the left and non-coronary cusps during diastole (figure 4.3). The left atrial diameter is measured in the same 2DE frame (i.e in diastole also) by extending a line across the left atrium in a continuation of the line used to measure the aorta. This measurement generally does cross the body of the left atrium, which is not generally possible in dogs with conventional M-mode cursors. This probably offsets the reduction in dimension one would expect with a diastolic frame, so that the LA:Ao ratio is of a similar magnitude in normal dogs as for M-mode ratios ( $1.0 \pm 0.06$  for normal Cavalier King Charles spaniels) (Haggstrom *et al.*, 1994). Left atrial areas are also reported for normal Dobermanns by Minors and O'Grady, who employed planimetry to measure left atrial areas from right parasternal long axis views (Minors and O'Grady, 1998).

#### 1.4 DOPPLER ECHOCARDIOGRAPHY

Ultrasound was applied to the measurement of blood flow velocity as early as 1970, using the Doppler principle that waves reflected by a moving target undergo a change in frequency that is directly related to the velocity of the reflector (Baker, 1970). The role of Doppler echocardiography in the measurement of the direction,

velocity and timing of blood flow within the heart has subsequently assumed primary importance in clinical echocardiography.

#### 1.4.1 Principles of Doppler echocardiography

In Doppler echocardiography, the transducer acts as both transmitter and receiver (as with M-mode and 2DE). Doppler echocardiography is based on the Doppler effect, which is expressed by the Doppler equation:

Formula 1.1

$$\text{Doppler shift } (\Delta f) = f_1 - f_2 = f_1 (2v \times \cos \theta / c)$$

where  $f_1$  = transmitted frequency,  $f_2$  = received frequency,  $c$  = velocity of sound in blood,  $v$  = velocity of the received signal, and  $\theta$  = the angle of the transmitted wave to the moving object.

The Doppler effect is used in Doppler echocardiography whereby ultrasound waves reflected by moving red blood cells demonstrate a shift in frequency. If the frequency of the transmitted wave and the velocity of sound in blood are known, then the velocity of the moving red blood cells can be calculated. As the shift in frequency is related to  $\cos \theta$ , it is important that the angle of interrogation is close to  $0^\circ$  (or at least less than  $20^\circ$ ). At angles greater than  $20^\circ$ , the shift in frequency will be significantly less and the velocity of blood flow will be underestimated.

Doppler echocardiography signals may be displayed as spectral Doppler, where a graph is shown of blood flow velocity against time. Concurrent 2DE images

allow placement of the cursor in specific sites within the heart. With continuous wave Doppler, two elements are used, with one acting as a continuous transmitter and the other as a continuous receiver. This format allows high velocity flow to be represented, but "range ambiguity" is introduced, as velocities are displayed from a range of tissue depths. In pulsed wave (PW) Doppler, the ultrasound waves are transmitted as pulses of waves, allowing the interrogation of blood flow velocities within a specific distance from the transducer. The distance is represented as a sample volume on the cursor. The maximum velocities that can be displayed without ambiguity by PW Doppler are limited. High velocities result in "aliasing", where blood flow will be displayed as both positive and negative velocities (ie, wrapping around the baseline). The velocity at which aliasing will occur depends on the Nyquist limit of the particular system. A compromise is a form of pulsed Doppler that uses multiple sample volumes: high pulse repetition frequency Doppler (HPRF). Frequent pulses of ultrasound waves are produced, so that a number of sample volumes will be superimposed on the 2D image. This allows the display of higher velocities without aliasing, but also increases the number of possible sites from which the velocities are being recorded.

Colour flow Doppler echocardiography represents the velocity and direction of blood flow in colour, superimposed on a black-and-white two-dimensional image. In effect, the colour is displayed within a very large sample volume superimposed on the 2D image. The accepted convention for depiction of blood flow direction is red towards the transducer, and blood flow away from the transducer is shown in blue. Disturbed or turbulent flow may be displayed in green or yellow, depending on the

internal algorithms used within the particular ultrasound machine. Aliasing may also occur in colour flow Doppler echocardiography.

#### 1.4.2 Doppler echocardiography in the assessment of systolic function

##### *1.4.2.1 Aortic velocity and acceleration*

Following Rushmer's descriptions of the value of the "initial ventricular impulse" in the evaluation of ventricular performance (Rushmer, 1964), early investigators recognised the potential applications of Doppler echocardiography in measuring aortic velocity and acceleration (Light, 1969; Sequeira *et al.*, 1976). Rushmer proposed that the initial ventricular "impulse" (defined as the product of force and time) could be used as a measurement of ventricular performance, as this variable was sensitive to changes in inotropic state. The initial ventricular impulse was increased during exercise, sympathetic stimulation, and following long diastolic intervals. A depressed initial impulse was seen with acute coronary occlusion, exsanguination hypotension, premature ventricular impulses, and general anaesthesia. Rushmer used implanted pulsed ultrasonic flowmeters around the aortic root to demonstrate these effects, asserting that peak aortic flow acceleration ( $dV/dt$  max) reflected the magnitude of the initial ventricular impulse (Rushmer *et al.*, 1963).

Van den Bos and co-workers (1973) used electromagnetic flowmeters implanted around the aorta in dogs to compare  $dV/dt$  max with more widely used indices of left ventricular systolic function, such as the maximum rate of rise of left ventricular pressure (LV  $dP/dt$  max). They considered a satisfactory measure of

contractile function to be one that changed less than 10% with changes in ventricular filling or aortic pressure, as most of the variables they examined changed by only 10-20% with known inotropic interventions. Neither  $dV/dt$  max nor any of the more traditional isovolumic pressure-based variables fulfilled these criteria. Nevertheless, Noble and others (1966) demonstrated that  $dV/dt$  max was more sensitive to changes in LV function than LV  $dP/dt$  max following coronary occlusion and intracoronary infusions of isoprenaline and calcium, and Lambert and others (1983) also suggested that  $dV/dt$  max was less preload- and afterload-dependent than LV  $dP/dt$  max.

It was only with the advent of transcutaneous Doppler ultrasound probes that these techniques became applicable to human patients (Light, 1969; Sequeira *et al.*, 1976). Kolettis and others (1976) were unable to distinguish patients with good, moderate and poor LV function using peak aortic velocity and  $dV/dt$  max, although the correlation with stroke volume was good.

#### *1.4.2.2 Cardiac output*

Another attractive potential application of Doppler echocardiography for those seeking to assess systolic function noninvasively was the ability to calculate stroke volume and cardiac output. Integration of the aortic velocity spectral Doppler envelope (velocity time integral) yields stroke distance, and multiplying this by the aortic area gives stroke volume. Cardiac output is the product of stroke volume and heart rate. In an open-chest dog model, Steingart and co-workers (1980) compared values for the aortic velocity time integral using pulsed Doppler echocardiography with stroke volume obtained with an electromagnetic flowmeter, obtaining correlation coefficients ranging from 0.74 to a very respectable 0.96. Fisher and



others (1983) also used an open-chest canine model to examine the effects of sample volume placement on Doppler calculation of cardiac output, using a roller pump system to vary cardiac output at known values. Angle correction factors were used, but the cross-sectional area of the aorta was calculated from 2DE images. Correlations were better ( $r = 0.98-0.99$ ), with standard errors of the estimates of only 0.2 L/min. Variations in sample volume size and position did not appear to alter the correlation appreciably.

Validating the technique in human patients was more problematic, owing to a lack of a suitable comparison technique. Cardiac output can be measured invasively using a thermodilution technique, or the Fick oxygen method. Each of these techniques has an error rate of up to 10% (Grossman, 1996; Ihlen *et al.*, 1984). Potential sources of error using Doppler echocardiographic techniques to calculate cardiac output include poor alignment with flow leading to underestimation of stroke distance; inaccurate measurement of the cross-sectional area; variation in cross-sectional area during the flow period; and choice of measurement of modal or maximum velocity for the spectral envelopes.

Ihlen and others (1984) found the best correlations with invasive techniques when the maximum velocity in the aortic root and the aortic orifice area were used in the calculations. This site was also preferred by Stewart and co-workers (1985). Certainly better results were obtained when 2DE rather than M-mode was used to measure aortic diameter, as one would expect (Gardin *et al.*, 1985). Zoghbi and Quinones (1986) advocated tracing the maximum velocity because of improved reproducibility, and it has been suggested that the maximal velocity signals may be less sensitive to poor angulation than modal velocity signals (Gisvold and Brubakk,

1982). The modal velocity has been proposed by other authorities because of good correlations with invasive techniques (Goldberg *et al.*, 1988). Loeber's group (1987) established that changes in aortic area during the cardiac cycle were of the order of  $9 \pm 2\%$ , and this did not change under a range of different loading conditions and inotropic states in open-chest dogs.

The velocity time integral alone has been used by some workers to assess stroke volume, eliminating the need for the problematic cross-sectional area measurement (Haite *et al.*, 1985). Measurement of stroke distance appeared to be adequately reproducible in vitro and in human patients, although offered no real advantage over cardiac output apart from ease of measurement (McLennan *et al.*, 1986).

#### *1.4.2.3 Systolic time intervals*

Systolic time intervals can also be obtained from Doppler recordings of aortic flow, with a concurrent ECG (Darke *et al.*, 1993; Koito and Spodick, 1989; Minors and O'Grady, 1998; Sequeira *et al.*, 1976).

### **1.4.3 Doppler echocardiography in the assessment of diastolic function**

Although initial excitement about the evaluation of ventricular performance with Doppler echocardiography centred on systolic function, this has subsequently been overshadowed by its increasingly central role in the assessment of diastolic function. The assessment of diastolic performance has always been fraught with

difficulties, and has traditionally relied on invasive techniques for any objective evaluation. Diastolic function can be considered to consist of two main components: left ventricular relaxation, and left ventricular chamber compliance. Chamber compliance is affected by myocardial tissue characteristics (e.g. increased interstitial fibrosis), chamber dimensions (e.g. ventricular hypertrophy), and operating compliance. Operating compliance is the change in chamber stiffness that occurs depending on the degree of filling of the chamber. At higher diastolic volumes the ventricle is pushed onto a steeper part of the end-diastolic pressure-volume relationship, so that for a given increase in volume there is a bigger increase in pressure. In addition, atrial function, heart rate and loading conditions may all have an effect.

Invasive techniques used to assess relaxation include measurement of the peak negative change of left ventricular pressure (peak negative  $dp/dt$ ), and the time constant of isovolumic pressure decline ( $\tau$ ), which is calculated as the inverse slope of the linear relation of the natural log of pressure versus time (Weiss *et al.*, 1976). Chamber compliance is generally assessed by evaluating the diastolic pressure-volume relationship (Gilbert and Glantz, 1989). With left ventricular pressure plotted on the ordinate axis and volume on the abscissa, abnormal diastolic function generally moves the diastolic pressure-volume relation upward and/or leftward.

Doppler echocardiography can be used to record the velocity of blood flow across the mitral valve during systole, documenting rapid early filling (mitral E wave) and additional flow in late diastole following atrial contraction (mitral A wave). Early studies in normal human subjects compared Doppler velocities of filling with radionuclide studies, and found good agreement in the percentage of

atrial contribution by the two techniques, but no significant relationship between Doppler peak filling rate and radionuclide peak filling rate (Pearson *et al.*, 1988). In the study by Pearson and co-workers (1988), the Doppler peak filling rate was calculated as the product of the peak E wave velocity and the cross-sectional area of the mitral valve, assuming a circular orifice geometry, whereas the percentage atrial contribution was derived entirely from the velocity time integral tracings. It has been shown in dogs that the mitral orifice is best approximated by an elliptical model, and the geometric error in calculating transmitral flow for peak filling rate might account for the poorer correlation than with the percentage atrial contribution, in which no such assumptions were made.

#### *1.4.3.1 Doppler evaluation of diastolic function in canine models*

It might be expected that a comparison between Doppler transmitral flow methods and radionuclide studies might show poor agreement, as the two methods evaluate different aspects of ventricular filling. As a consequence, a number of studies were carried out in dogs in an attempt to elucidate the factors influencing transmitral flow. Nishimura and others (1989) examined the effects on transmitral flow of varying preload and afterload in closed chest dogs with atrial pacing. They found significant changes with preload in peak E and A velocities, mitral E deceleration times, and the ratio of early to late filling (E/A), and variable effects with increased afterload (depending on prevailing filling pressures). They concluded that no Doppler transmitral variable should be equated directly with traditional invasive measures such as the time constant of relaxation ( $\tau$ ), filling pressures, or

chamber compliance, but that certain transmitral patterns emerged under particular haemodynamic conditions.

Yellin (1990) stressed the importance of the left atrial-ventricular pressure gradient in determining the pattern of transmitral flow. By implanting atrial and ventricular micromanometers in dogs, along with electromagnetic flowmeters to measure transmitral flow, he demonstrated that increases in left atrial pressure could overcome the effects of decreased rates of relaxation to result in normal flow patterns.

An additional diastolic variable that could be measured using Doppler echocardiography was the isovolumic relaxation period (IVRT). Myreng and Smiseth (1990) compared the isovolumic relaxation period (measured from aortic valve closure noise to early mitral opening signal) with  $\tau$  (measured from high-fidelity left ventricular pressure tracings). In their open-chest dog studies they found a significant correlation between IVRT and  $\tau$  at normal filling pressures, but IVRT did not show a corresponding increase with  $\tau$  when left atrial pressures were increased. Appleton and others (1991) examined the effect of heart rate on transmitral filling by three different methods in lightly sedated dogs, and showed no significant effect on E wave velocity. With moderate increases in heart rate there was an initial decrease in A wave velocity, whereas A wave velocity increased at higher rates, as shorter diastolic periods led to encroachment on early diastole by atrial contraction, and E and A waves began to summate. Similar findings were reported by Yamamoto and co-workers (1993a) in dogs with atrial pacing.

From all these studies it was evident that transmitral Doppler flow patterns were the result of an interplay of numerous different forces, including left ventricular relaxation, left ventricular chamber compliance, left atrial pressures and compliance, and heart rate. Despite the fact that transmitral Doppler flow patterns failed to give any pure value of diastolic function, they were avidly adopted by clinicians wishing to evaluate diastolic function in clinical patients.

#### *1.4.3.2 Evaluation of diastolic function in human subjects by DE*

The lack of a noninvasive technique for assessing diastolic function meant that its study had largely been restricted to the laboratory and research settings. It was little wonder that reports quickly appeared of the use of transmitral Doppler flow patterns in a number of human cardiac diseases, including hypertrophic cardiomyopathy (Iwase *et al.*, 1987; Maron *et al.*, 1987; Takenaka *et al.*, 1986a), coronary artery disease (Wind *et al.*, 1987), and dilated cardiomyopathy (Appleton *et al.*, 1988b; Takenaka *et al.*, 1986b).

Some early reports were somewhat simplistic, with abnormal diastolic function often being equated with abnormal chamber compliance (Gardin, 1987; Kitabatake *et al.*, 1982). A reversal of the normal ratio of early to atrial diastolic filling (i.e., reduced early filling with enhanced filling following atrial contraction) was commonly recognised in patients with heart disease, and this was frequently ascribed to abnormal chamber compliance (Gardin, 1987; Kitabatake *et al.*, 1982). By studying Doppler filling patterns and simultaneous invasive pressure measurements in human patients with coronary artery disease, Stoddard and co-workers (1989b) were able to show that delayed relaxation and increased chamber

stiffness actually had contrasting effects on Doppler transmitral velocities. Delayed relaxation caused a decrease in early filling and an increase in atrial filling, whereas increased chamber stiffness resulted in enhanced early filling and decreased atrial filling (Stoddard *et al.*, 1989b).

The influence of age on human transmitral flow patterns was not initially recognised, but a negative correlation between mitral E/A ratio and age has been consistently found in many studies (Benjamin *et al.*, 1992; Manca *et al.*, 1992; Voutilainen *et al.*, 1991). Even in populations rigorously screened for heart disease such as the Framingham study, transmitral filling patterns were found to change dramatically with age, and to be independent of left atrial and ventricular dimensions (Benjamin *et al.*, 1992). Benjamin and others (1992) postulated that these changes were caused by an age-related reduction in left ventricular distensibility, but one would expect an opposite effect on mitral E/A ratio if this were the predominant change with age. A more likely explanation is that a reduction in the rate of left ventricular relaxation accounts for the shift in filling towards late diastole.

Appleton and others (1988b) showed that a range of abnormal transmitral patterns might be observed in patients with diastolic dysfunction, and that these patterns often reflected haemodynamic status rather than the underlying cause of cardiac disease. In patients with coronary artery disease and dilated cardiomyopathy but low left atrial pressures (as assessed by pulmonary capillary wedge pressures), the typical pattern seen was a normal or reduced mitral E/A ratio, and normal or prolonged mitral E wave deceleration time and isovolumic relaxation time. In patients with higher filling pressures, usually the isovolumic relaxation time and mitral deceleration times were short, and the E/A ratio was increased, whether the

patient had coronary artery disease, dilated cardiomyopathy, or a restrictive myocardial process. This was confirmed as a common transmitral flow pattern in a further study of patients with restrictive physiology demonstrated by catheterisation (i.e. abrupt termination of left ventricular filling, resulting in a “dip and plateau” configuration on left ventricular pressure tracings, and elevated end-diastolic pressures) (Appleton *et al.*, 1988a).

Appleton and Hatle (1992) proposed that the variation in transmitral flow patterns reflected different stages of a progression common to many cardiac disease states. The earliest stage (“abnormal relaxation”) was consistent with delayed ventricular relaxation but normal filling pressures (reduced mitral E/A ratio); as left ventricular chamber stiffness and left atrial pressures increased, the mitral E/A ratio would normalise despite the persistence of delayed relaxation (“pseudonormal”); the final change was associated with further increases in chamber stiffness and left atrial pressure, leading to an abrupt end in early ventricular filling and a diminished A wave (“restrictive”). Evidence for this was supported by studies in human patients with cardiac amyloid disease, where mildly affected (early) cases had evidence of a delayed relaxation pattern, and severely affected (advanced) cases had a restrictive pattern (Klein *et al.*, 1990). A canine rapid ventricular pacing model finally confirmed that this progression in diastolic filling patterns does indeed take place with progressive myocardial dysfunction (Ohno *et al.*, 1994).

The influence of preload has confounded the interpretation of transmitral flow patterns, with reports of decreased preload leading to abnormal relaxation patterns, and increased left atrial pressures masking abnormal relaxation patterns. Although it has been suggested that decreased preload can lead to a fall in E wave



velocities and no change or an increase in A wave velocities, this may only be the case in older human subjects, where reducing preload will reveal a delayed relaxation pattern in place of a previously pseudonormal one (Choong *et al.*, 1987; Stoddard *et al.*, 1989a). One study in dogs showed a fall in both E and A wave velocities with a reduction in preload, with the E/A ratio actually increasing (Nishimura *et al.*, 1989). Reducing preload using a Valsalva manoeuvre resulted in a decrease in E/A ratio in patients with coronary artery disease, but minimal change in E/A ratio in normal subjects in another study (Dumesnil *et al.*, 1991), and a Valsalva manoeuvre is now considered one of the acceptable methods of unmasking “pseudonormal” filling patterns (Oh *et al.*, 1999a; Rakowski *et al.*, 1996).

#### *1.4.3.3 Newer diastolic applications of Doppler echocardiography*

In view of the many influences on transmitral filling patterns, considerable effort has been devoted to finding additional or alternative techniques for extracting more objective information about diastolic function using Doppler echocardiography. Considerable strides have been made over the past five years. Interrogation of pulmonary venous flow velocities led to a significant advance in assessment of filling pressures. Pulmonary venous flow reflects changes in left atrial pressure, and three main phases can usually be identified: forward systolic flow, forward diastolic flow, and retrograde flow with atrial contraction (Rossvoll and Hatle, 1993). The velocity and duration of this atrial reversal wave appears to be particularly useful in identifying elevated left ventricular end-diastolic pressure (Appleton, 1997; Klein *et al.*, 1998; Yamamoto *et al.*, 1997).

Doppler tissue imaging has been another new development (McDicken *et al.*, 1992; Sutherland *et al.*, 1994). Myocardial velocities are displayed in place of blood flow velocities, in either a colour-coded two-dimensional, M-mode or pulsed Doppler format. Current applications in the assessment of diastolic function include measurement of mitral annulus velocities. In diastole, pulsed wave tissue Doppler of the mitral annulus results in an early (E') wave and an atrial (A') wave, which parallel the mitral flow velocities. The E' wave velocity is reduced with abnormal relaxation, but does not increase with restrictive filling patterns, in contrast with the mitral E wave. Sohn and others (1997) showed that this feature therefore makes mitral annular velocities helpful in distinguishing pseudonormal from normal diastolic filling patterns. The ratio of mitral E velocity / mitral annular E' velocity appears to be one of the best predictors of pulmonary capillary wedge pressures of all the available Doppler indices, with one study showing a correlation coefficient of 0.87 ( $p < 0.001$ ) even when mitral E and A waves were fused (Nagueh *et al.*, 1998).

Colour M-mode Doppler echocardiography has also been applied to the assessment of diastolic function, when used to assess flow propagation within the left ventricle (Brun *et al.*, 1992). If the M-mode cursor is aligned with mitral inflow in an apical four-chamber view, the velocity of flow propagation can be measured as the slope of the colour wave of early filling (Takatsuji *et al.*, 1996). The velocity of flow propagation is currently attracting much interest because of its relative preload-independence (Garcia *et al.*, 1999).

#### 1.4.4 Doppler assessment of valvular regurgitation

The severity of mitral regurgitation has been traditionally assessed by angiographic methods. The severity of regurgitation can be assessed using a qualitative technique based on left ventricular injections of contrast and subsequent opacification of the left atrium, with 1+ representing mild regurgitation and 4+ representing severe regurgitation. A quantitative technique can also be used by incorporating a measure of cardiac output, such as with a thermodilution technique. This gives a value for forward stroke volume. The total stroke volume is measured by subtracting the left ventricular end-systolic volume from the end-diastolic volume. The regurgitant fraction is the difference between the total stroke volume and the forward stroke volume, divided by the total stroke volume.

Similar principles have been applied to Doppler echocardiography. A qualitative method (spatial mapping) is the Doppler technique that correlates best with the qualitative angiographic equivalent (Spain *et al.*, 1989; Wu *et al.*, 1993). In spatial mapping, the jet area is related to the size of the left atrium. Compared to angiographic detection of mitral regurgitation, sensitivity and specificity of colour flow Doppler was found to be excellent (100% and 100%, respectively) (Helmcke *et al.*, 1987). However, accurate assessment of jet size requires three orthogonal views to minimise underestimation of eccentric jets, and the jet dimensions may also be affected by gain settings (Helmcke *et al.*, 1987). Spatial mapping correlates less well with quantitative angiography techniques, but has been widely adopted because it is simple, and the clinical significance of the different colour Doppler grades of mitral regurgitation is readily equated with the familiar angiographic grades.

Other quantitative Doppler techniques are available, such as a volumetric technique based on cardiac output and valvular flow (as with the quantitative angiographic technique) (Rokey *et al.*, 1986). The regurgitant volume (the difference between transmitral flow and aortic flow) can be used to calculate the effective regurgitant orifice when divided by the velocity time integral of the mitral regurgitant jet.

A newer technique, the proximal isovelocity surface area method (PISA) also yields the effective regurgitant orifice area (Enriquez-Sarano *et al.*, 1994; Enriquez-Sarano *et al.*, 1995a; Recusani *et al.*, 1991). As blood converges towards the mitral valve from the ventricular side during regurgitation, it forms flow convergence regions comprising multiple hemispheres of equal velocities. The flow rate at each isovelocity shell is equivalent to the flow rate at the regurgitant orifice, and one can discern an isovelocity shell by measuring the hemisphere at which aliasing takes place (it is usually necessary to adjust the Nyquist limit). The flow rate is calculated as the product of the area of the hemisphere and the aliasing velocity, and the effective regurgitant orifice is calculated as the flow rate divided by the velocity time integral of the mitral regurgitant jet. These quantitative Doppler techniques correlate well with quantitative angiography and with each other, but only poorly with qualitative angiography (Blumlein *et al.*, 1986; Dujardin *et al.*, 1997a; Enriquez-Sarano *et al.*, 1995b; Keren *et al.*, 1988a; Rokey *et al.*, 1986). This is not necessarily a failure of the quantitative Doppler techniques, but their widespread adoption will require a change in the perception of mitral regurgitation, from dependence on qualitative grades to use of quantifiable variables, such as regurgitant fraction.

#### 1.4.5 Veterinary applications of Doppler echocardiography

Doppler echocardiography is a relatively new diagnostic modality in veterinary medicine, although there are a number of reviews of the subject in the veterinary literature (Bonagura and Miller, 1998a; Bonagura and Miller, 1998b; Darke, 1992; Gaber, 1991). Optimum views for recording Doppler velocities have been reviewed. (Darke *et al.*, 1993; Yuill and O'Grady, 1991). The optimum window for pulmonary artery velocities is generally obtained from a left cranial parasternal view, although the right parasternal window is almost as good. Aortic velocities are consistently higher from a subcostal window, although this view is more difficult to obtain in many dogs, and the signal strength is often weak. Mitral valve velocities are generally obtained from an apical two-chamber or four-chamber view, and tricuspid velocities from a modification of a left apical view.

There are still relatively few reports of reference intervals for normal canine Doppler blood flow velocities. Gaber (1987) was amongst the first to report normal values for Doppler blood flow in the veterinary literature. She separated her dogs into animals less than 10 kg in bodyweight, and those over 19 kg. She found no difference in aortic or pulmonary artery velocities between the groups, but found increased mitral and tricuspid E wave velocities in the larger dogs. This difference in E wave velocities in dogs of different sizes has not been reported in any other subsequent studies, although no other reports have specifically examined the influence of bodyweight on Doppler velocities.

Further reports of normal values followed from Brown and others (1991) for aorta and pulmonary artery only, and Yuill and O'Grady (1991). The latter workers

reported mitral and tricuspid values as single peak velocities only, although they differentiated inflow velocities into E and A waves in one of the accompanying illustrations. Their peak inflow velocities are higher than in many other reports, and it is possible that some of their animals had fused E and A waves owing to high heart rates (no heart rates are reported, and these dogs were unsedated). The velocities for fused E and A waves are generally higher than for normal E waves (Appleton *et al.*, 1991). Sedation (acepromazine) was used in the study by Brown and co-workers (1991) (Brown *et al.*, 1991). Bonagura and Miller (1998a) have subsequently reported on the effects of acepromazine, and a combination of acepromazine and buprenorphine on normal Doppler velocities. Aortic and mitral valve E wave velocities appeared to be unaffected by these sedation protocols, but pulmonary artery, mitral A wave and tricuspid A wave velocities were significantly reduced.

Kirberger and others (1992) reported normal values in young beagles and German shepherd dogs. His velocities were notably higher than those reported in other studies. This almost certainly reflects over-estimation of velocities resulting from his use of angle correction, which is not only unnecessary, but often an additional source of error (Goldberg *et al.*, 1988). Age has a very prominent influence on human mitral inflow values, yet it appears that only one report has evaluated the effect of age in dogs (Vandenberg *et al.*, 1990). In this study of a total of 11 dogs, young beagles (mean age 12.4 months) were compared with old beagles (mean age 137 months). No differences were found in mitral E or A wave velocities, nor in mitral E:A ratio, although the velocity time integral of the A wave was increased in the older dogs. This numbers of dogs in this study are probably too

small to be able to resolve the question of age influence in dogs adequately, but the effect does not appear to be as striking as in older people.

Some breed-specific values have been reported: Minors and O'Grady (1998) reported peak aortic and mitral inflow velocities, as well as aortic VTI and acceleration. These were compared in "normal" Dobermanns and Dobermanns considered to have occult DCM (Minors and O'Grady, 1998). Sottiaux and Amberger (1997) also reported peak aortic, pulmonary artery and mitral velocities in asymptomatic Dobermanns, although they included recordings from Dobermanns suspected to have occult DCM in their final values. Unfortunately they did not describe separate mitral E and A waves. Pietra and others (1998) reported Doppler velocities in normal English setters, and unsurprisingly, found no relationship with bodyweight in this narrow range of animals.

Overall, Doppler velocity values seem relatively consistent across species, with values in cats, horses and sheep not differing greatly from velocities reported in dogs and human beings (Blissitt and Bonagura, 1995; Domanjko and Thomas, 1996; Kirberger and Vandenberg, 1993; Reef *et al.*, 1989).

Of the newer Doppler indices, pulmonary venous flow velocities have been reported in dogs (Schober *et al.*, 1995; Schober *et al.*, 1998) and in cats (Santilli and Bussadori, 1995; Santilli *et al.*, 1996). Pulsed wave tissue Doppler velocities have been reported in normal and cardiomyopathic cats (Gavaghan *et al.*, 1998).

## 1.5 ULTRASONIC TISSUE CHARACTERISATION

### 1.5.1 Interaction of ultrasound waves with biological tissue

Ultrasonic tissue characterisation is based on the assumption that the morphology of a biological tissue will affect its acoustic properties. Ultrasound waves will be reflected by boundaries between tissues of different acoustic impedance, a property that is influenced by tissue density and elasticity (McDicken, 1991a). Ultrasound waves will also interact with tissue in different ways according to the size, orientation and distribution of scatterers within the tissue. Conventional diagnostic echocardiography relies heavily on specular reflections, which are produced when ultrasound waves are reflected by interfaces of large acoustic impedance mismatch and dimensions exceeding the wavelength of the incident beam (McDicken, 1991b). Examples of myocardial specular reflections are the endocardial and epicardial surfaces, and valve leaflets. Specular reflections are angle-dependent, so that the endocardium acts as a specular reflector only in views where the incident wave is perpendicular to the endocardial surface. Ultrasound waves behave differently when reflected by scatterers that are smaller than the incident wavelength. Sound waves are reflected in multiple directions by small scatterers ("Rayleigh scatter"), and the proportion of waves reflected back to the transducer is not angle-dependent (McDicken, 1991b). However, these "backscattered" waves are of low intensity compared with specular reflections. For example intramural myocardium gives rise to backscattered echoes, whereas the pericardium and endocardium produce specular echoes (when perpendicular to the incident beam). Although conventional echocardiography has been applied to the assessment of myocardial tissue architecture using analysis of echodensity and echotexture (Bhandari and Nanda,



1983), this information is qualitative and subject to influence from both equipment and operator .

Quantitative information about the acoustic properties of the myocardium can be gathered in a number of ways. The principal techniques used in myocardial tissue characterisation have included *in vitro* attenuation studies, *in vitro* and *in vivo* integrated backscatter measurements, acoustic microscopy, mean grey scale techniques, and pattern and textural analysis. Only attenuation studies and integrated backscatter techniques will be considered here.

#### *1.5.1.1 Attenuation*

*In vitro* attenuation studies require the tissue in question to be placed in a water bath between a transmitting transducer and a receiving transducer. The signal received by the second transducer with a measured thickness of tissue in place is compared to the signal received with the same thickness of a fluid of known attenuation in place of the tissue (Miller *et al.*, 1976). Attenuation of the signal occurs as a result of absorption, reflection, refraction and scattering by the tissue, as well as wavefront divergence. Attenuation depends on the frequency of the ultrasound wave, and a graph can be plotted of amplitude of the received signal against frequency. The difference between the curves of amplitude against frequency (with and without the tissue) allows a *tissue attenuation coefficient* to be calculated as a function of frequency. Attenuation can be reported as the slope of a least squares line fit to the attenuation coefficient as a function of frequency, which is relatively independent of energy losses due to specular reflection (Miller *et al.*, 1976).

Attenuation techniques cannot be employed *in vivo* however, so they have been less useful in clinical applications.

#### 1.5.1.2 Backscatter

*In vitro* backscatter techniques are similar to attenuation studies, except that the transmitting transducer also acts as the receiver for ultrasound waves reflected back from the tissue. The interrogated tissue reflects pulses of echoes of different frequencies, and the power spectrum of this tissue is then compared with the power spectrum from a near-"perfect" reflector, such as a steel plate (Miller *et al.*, 1983). This allows the *backscatter transfer function* to be calculated, which is described as the efficiency with which ultrasound is backscattered at each frequency. *Integrated backscatter* is defined as the frequency average of the backscatter transfer function (Miller *et al.*, 1983).

Integrated backscatter (IB) is one of the most commonly employed indices in ultrasonic tissue characterisation. The method of calculating IB described above is termed *frequency domain* analysis, and this methodology has been used by a few groups (Cohen *et al.*, 1982; Sagar *et al.*, 1987). Frequency domain analysis is accurate but time-consuming, and involves Fourier transform techniques. Integrated backscatter can also be calculated in the *time domain* (Goens *et al.*, 1996; Lattanzi *et al.*, 1987; Masuyama *et al.*, 1989a; Moran *et al.*, 1994; Naito *et al.*, 1995; Rijsterborgh *et al.*, 1991; Thomas *et al.*, 1989; Zuber *et al.*, 1999). With time domain analysis, IB is estimated by squaring and summing the time-domain signal, and referencing this to the squared and summed time-domain signal from a near-perfect

reflector. Comparisons of both techniques using the same cardiac cycles have shown that results are comparable (Rijsterborgh *et al.*, 1993).

Although the technique for calculating IB in the time domain is potentially applicable to commercial echocardiographs, most machines do not allow access to the raw unprocessed radiofrequency (RF) signals. Conventional diagnostic echocardiographic machines employ extensive processing algorithms to enhance the visual appearance of the video image. Log-compression techniques and the restricted number of grey scales available in the final video image reduce the useful information available in the accessible signal from most machines. In contrast, the unprocessed RF signals offer more direct information on a linear scale with a wide dynamic range.

Absolute levels of IB require calibration of the signal against a perfect reflector, such as a steel plate. The effects of chest wall attenuation on IB are circumvented in open chest animal studies where the transducer is coupled to the epicardium. Closed chest studies are more problematic, and accurate estimates of IB are difficult to achieve. Methods of calibration used include referencing the signal from the region of interest to the pericardial signal (Lattanzi *et al.*, 1987), and using the Doppler power signal backscattered from blood (Naito *et al.*, 1995).

Many factors have been proposed as influencing the way in which the myocardium backscatters ultrasound waves, including myocardial blood flow, haematocrit, collagen, myocardial water content, and tissue architecture.

### *1.5.1.3 Effect of blood flow and haematocrit on attenuation/backscatter*

*In vitro* studies of myocardium from dogs following coronary occlusion showed an early fall in myocardial attenuation within the first 24 hours (Mimbs *et al.*, 1979). In contrast, the same group measured IB in a series of studies in canine hearts following coronary occlusion, and demonstrated an early increase in IB (1981). Areas exhibiting increased IB corresponded with areas of decreased myocardial blood flow, although this relationship was not linear, and abnormal IB was only seen when blood flow was <20% of control areas. Further studies in perfused rabbit hearts revealed that IB levels increased when the myocardial tissue was perfused with buffer solution, but returned to normal when perfused with whole blood, suggesting that whole blood constituents within the myocardial vasculature might be responsible for normal IB properties (Mimbs *et al.*, 1981). This was not supported, however, by a study by Haasler (1993) where haematocrit was reduced by fluid infusion, with no effect on IB.

A number of studies have demonstrated an increase in myocardial IB with acute ischaemia, often within minutes of coronary occlusion (Rasmussen *et al.*, 1984; Rijsterborgh *et al.*, 1990; Sagar *et al.*, 1990a; Sagar *et al.*, 1987), and there has been debate over whether this increase is related to a decrease in intravascular blood volume, myocardial oedema, inflammatory infiltrate, or decreased wall thickness. In Haasler's study (1993), where open chest dogs had infusions of adenosine as well as crystalloids, coronary blood flow and myocardial water content increased with no effect on IB. Rijsterborgh's group (1990) found no difference in end-diastolic IB values between acutely ischaemic and normal myocardium in pigs, although differences were seen in end-systolic values. As there is a concurrent decrease in

systolic wall thickness with ischaemia, it was suggested that this might be responsible for the increase in IB. Further studies by the same group examining the relationship between IB and wall thickness in pigs led them to conclude that wall thickness was a primary determinant of IB, and they showed that an increase in intravascular volume caused by increased perfusion pressure did not result in changes in IB (Rijsterborgh *et al.*, 1991; Rijsterborgh *et al.*, 1996).

A recent study by O'Brien and others (1995) in open chest dogs revealed differences in attenuation between normal myocardium and 'stunned' myocardium. Myocardial stunning occurs when tissue is subjected to brief ischaemia without resulting in irreversible changes. In O'Brien's study, IB was not abnormal in the stunned myocardium, but was abnormal in infarcted myocardium. The authors proposed that stunning was associated with biochemical changes without structural changes, and that this could influence attenuation without affecting IB. In contrast, infarction was associated with ultrastructural changes in the tissue architecture that affected IB as well as attenuation.

#### *1.5.1.4 Effect of myocardial water content on attenuation/backscatter*

The increase in IB seen with early myocardial ischaemia has been ascribed to an increase in myocardial water content (Mimbs *et al.*, 1981; Sagar *et al.*, 1990a). In an open chest dog model, IB and myocardial water content increased following acute myocardial ischaemia, and the authors proposed that increased myocardial water was the dominant tissue change responsible for increased IB and decreased attenuation (Sagar *et al.*, 1990b). Haasler's study (1993) with crystalloid infusion in

dogs did not support myocardial water being the dominant effect on increased IB in acute ischaemia, as they saw no change in IB despite increased interstitial oedema and intracellular water.

It is possible that increased myocardial water in acute ischaemia is responsible for a change in tissue attenuation, but not IB.

#### *1.5.1.5 Effect of collagen on attenuation/backscatter*

Early studies revealed an association between myocardial collagen and ultrasonic attenuation and backscatter. In normal hearts, higher levels of both collagen and IB were measured in the right ventricle compared with the left ventricle (Hoyt *et al.*, 1984).

Studies in canine and rabbit hearts following coronary occlusion showed that attenuation, integrated backscatter and collagen content all increased in infarct zones over time (Mimbs *et al.*, 1980). Although increased IB levels were associated with increased collagen as assessed by hydroxyproline assay, IB decreased when similar myocardial samples were perfused with collagenase, while attenuation remained the same. This finding suggests that intact collagen is an important determinant of IB, whereas effects on attenuation are similar whether collagen is intact or fragmented. Chronic myocardial infarction leads to marked fibrosis, and has been consistently associated with increased IB (Mimbs *et al.*, 1979; Mimbs *et al.*, 1977; Thomas *et al.*, 1989).

In both a rabbit doxorubicin cardiomyopathy model and in Syrian hamsters with cardiomyopathy, increased IB levels were measured in fibrotic areas or regions

of myocardium with increased collagen content (Mimbs *et al.*, 1981; Perez *et al.*, 1984). The resolution of in vitro IB techniques has improved with the advent of acoustic microscopy, which uses high frequency (50 MHz) ultrasonic interrogation to measure IB levels. The sensitivity of IB to conformational changes in collagen has led to new applications for acoustic microscopy in assessing myocardial remodelling and interstitial fibrosis. Acoustic microscopy in a tight-skin mouse model versus controls showed a good correlation with hydroxyproline assay and IB levels, despite no evident difference in the two groups using light microscopy (Wong *et al.*, 1993). Electron microscopy showed myocardial changes to consist of mild to moderate increase in interstitial fibrosis only. Acoustic microscopy has also been applied to captopril-treated cardiomyopathic Syrian hamsters, where investigators found that captopril decreased IB levels without altering collagen as measured by hydroxyproline assay (Davison *et al.*, 1994; Davison *et al.*, 1995). The authors concluded that a reduction in scar tissue calcification was responsible for the decreased backscatter, although alterations in collagen cross-linking could also affect backscattering properties.

### 1.5.2 Cyclic variation in integrated backscatter (CVIB)

Numerous studies have demonstrated consistent variation in integrated backscatter according to the phase of the cardiac cycle (Barzilai *et al.*, 1984; Glueck *et al.*, 1985; Madaras *et al.*, 1983; Milunski *et al.*, 1989a; Mohr *et al.*, 1989; Rhyne *et al.*, 1986; Wear *et al.*, 1989; Wickline *et al.*, 1985a; Wickline *et al.*, 1985b). In most studies, a maximum value for integrated backscatter was found at end-diastole, and a minimum value at end-systole. The magnitude of cyclic variation of IB (CVIB)

appears to vary according to the myocardial site studied, with apical views resulting in reduced or reversed cyclic variation (Lange *et al.*, 1995; Vandenberg *et al.*, 1989). At least part of this view-dependence may be related to effects from the angle of insonification, because of the anisotropic characteristics of myocardium (i.e., the properties vary with different orientation), just as absolute levels of IB are affected by the angle of insonification (Holland *et al.*, 1997; Holland *et al.*, 1998; Recchia *et al.*, 1995).

The distribution in CVIB within the ventricle and within the myocardial wall (from epicardial to subendocardial) parallels regional variation in contractile function (Colonna *et al.*, 1999; Miller *et al.*, 1983; Mottley *et al.*, 1984).

The timing of the variation in IB with respect to the cardiac cycle also appears to be important, and some measure of the 'phase delay' has been incorporated into assessment of cyclic variation by some workers (Mobley *et al.*, 1995; Mohr *et al.*, 1989).

#### *1.5.2.1 Determinants of cyclic variation*

CVIB has been found to be blunted by myocardial ischaemia, and to return on reperfusion (Barzilai *et al.*, 1984; Barzilai *et al.*, 1990; Glueck *et al.*, 1985; Sagar *et al.*, 1990). A restoration of normal CVIB was shown to precede the return of normal wall thickening following reperfusion in stunned canine myocardium (Milunski *et al.*, 1989c), and in a study evaluating CVIB immediately after revascularisation in human patients with coronary artery disease, CVIB indicated successful reperfusion three weeks before increased wall motion was observed



(Hirata *et al.*, 1999a). However, the relationship between CVIB and wall thickness remains unclear. In another study of normal human subjects, CVIB remained the same with dobutamine infusion despite an increase in wall thickening. Despite the apparent discordance between wall thickness and CVIB in these studies, Rijsterborgh and others (1996) showed that simulation of systole and diastole by distension of isolated hearts did not result in any appreciable change in wall thickness or CVIB. Following their earlier studies, where they found an inverse relationship between IB and wall thickness (Rijsterborgh *et al.*, 1991; Rijsterborgh *et al.*, 1990), they asserted again that wall thickness was a key determinant of IB, and that CVIB was related to variation in wall thickness over the cardiac cycle. However, a study in isolated canine papillary muscles showed that CVIB occurs in isotonicity contracting myocardium, but not isometric contraction (Wear *et al.*, 1986), which suggests that some form of geometric alteration of scatterers within the myocardium is probably responsible for CVIB.

Loading conditions do not appear to have a marked effect on CVIB, and two separate studies examining the effect of altering preload and afterload showed no change in CVIB (Naito *et al.*, 1996; Sagar *et al.*, 1988). In the same studies, cyclic variation increased with dobutamine and decreased with beta-adrenergic blockade, paralleling changes in contractile performance as assessed by LV dP/dt.

#### *1.5.2.2 Mechanism of CVIB*

The precise mechanism of this cyclic variation is unknown. It has been proposed that the intracellular and extracellular elastic domains are cyclically altered in diastole and systole, thereby leading to dynamic changes in local acoustic

impedance mismatch (Wickline *et al.*, 1985a). However, the studies by Wear and co-workers (1986) suggest that tissue elastic properties may be relatively unimportant, as tissue elastic properties should change in isometric contraction as well as in isotonic contraction. It seems plausible that CVIB is produced by an effective change in shape, area and orientation of the scatterers within the myocardium (Sagar *et al.*, 1988). Results of a recent study have suggested that variation in IB may be directly related to sarcomere length (O'Brien *et al.*, 1995). Rose and others (1995) proposed that the elementary scatterer within the myocardium is related to the collagen support that comprises myocyte bundles. Both theories would account for anisotropy, and both are consistent with the association between CVIB and geometric wall changes.

### 1.5.3 IB findings in cardiac disease

As in animal studies, cyclic variation has been found to be reduced in human patients with acute myocardial ischaemia, and with remote myocardial infarcts. A consistent improvement in CVIB has been demonstrated in human ischaemic segments following reperfusion, usually preceding a return of normal wall motion, and sometimes even when wall motion fails to normalise at all (Hirata *et al.*, 1999b; Iliceto *et al.*, 1997; Milunski *et al.*, 1989b; Takiuchi *et al.*, 1998; Vandenberg *et al.*, 1991). In addition, a delay in the nadir of cyclic variation has been observed in infarcted sites (Vered *et al.*, 1989).

Other pathological conditions in which abnormal ultrasonic tissue characteristics have been noted include acute allograft rejection (Angermann *et al.*, 1997; Masuyama *et al.*, 1990), doxorubicin toxicity (Goens *et al.*, 1999), diabetes

mellitus (Di Bello *et al.*, 1995; Perez *et al.*, 1992), aortic stenosis and aortic insufficiency (Di Bello *et al.*, 1997), and cyanotic heart disease in adults (Hopkins *et al.*, 1994). Abnormal CVIB has also been noted in patients with dilated cardiomyopathy (see chapter 6).

Ultrasonic tissue characterisation techniques have also been applied to myocardial hypertrophy. Integrated backscatter levels (normalised for the pericardial signal) were shown to be increased in patients with hypertrophic cardiomyopathy (HCM) when compared with normal controls, even in non-hypertrophied regions of myocardium (Lattanzi *et al.*, 1991). Using the same technique, integrated backscatter levels were found to be increased in patients with HCM compared with elite athletes with comparable degrees of left ventricular hypertrophy (Lattanzi *et al.*, 1992). The same investigators compared senior elite athletes with age-matched sedentary controls (Di Bello *et al.*, 1993). Despite increased wall thickness in the athletes, IB levels were similar in both groups. In another study, CVIB was decreased in the septum of patients with HCM and in patients with pressure overload hypertrophy, compared with normal controls (Masuyama *et al.*, 1989b). Lucarini and others (1994) found that in a study of treated hypertensive patients, there was no normalisation of IB following a reduction in LV mass and wall thickness, with IB remaining high.

These findings tend to suggest that increased IB levels may be a result of abnormal tissue structure in LV hypertrophy, and not simply a reflection of increased wall thickness. Increased myocardial fibrosis is seen in both hypertensive hypertrophy and hypertrophy associated with HCM, and collagen is known to have a marked effect on IB. Myofibre disarray is also found in HCM, and the hypothesis

that myocyte bundles may represent the main scattering unit in myocardium (Rose *et al.*, 1995) is consistent with these conditions resulting in increased IB.

The author is not aware of any reports of IB measurements in naturally-occurring cardiac disease in dogs.

## CHAPTER 2: STUDY SUBJECTS

### 2.1 INTRODUCTION

Although idiopathic dilated cardiomyopathy (DCM) has been reported in many breeds of dog, the form of DCM seen in both Dobermanns and English cocker spaniels is distinctive. This chapter outlines the specific reported features of DCM in Dobermanns and English cocker spaniels, and describes the clinical characteristics of the dogs reported in this study.

### 2.2 BACKGROUND

#### 2.2.1 Dobermann DCM

Dobermanns were amongst the earliest breeds reported with DCM (Ettinger and Suter, 1970b), and they have been consistently cited as one of the preponderant breeds in most subsequent reviews of canine DCM (Buchanan, 1992; Fox, 1988; Keene, 1989; Thomas, 1987; Wood, 1983) (Hazlett *et al.*, 1983; Lamontagne and DiFruschia, 1991; Monnet *et al.*, 1995). An analysis of the Veterinary Medical Database (VMDB) at Purdue University from 1986 to 1991 showed a prevalence in Dobermanns of 5.8%, which was second only to Scottish deerhounds (6.0%) (Sisson and Thomas, 1995). Of 260 dogs diagnosed with DCM between 1986 and 1996 at the University of California, Davis Veterinary Medical Teaching Hospital, 33% were Dobermanns, constituting the most commonly affected breed (Kittleson, 1998).

It is widely recognised that Dobermanns suffer a clinically distinct form of DCM, with a particularly poor prognosis compared with other breeds (Fox, 1988; Lamontagne and DiFruschia, 1991; Sisson and Thomas, 1995). Dobermann DCM has

been the subject of specific study by a number of groups; principally Calvert's group at the University of Georgia (Calvert, 1984; Calvert, 1986; Calvert, 1991a; Calvert, 1991b; Calvert, 1991c; Calvert, 1991d; Calvert, 1991e; Calvert, 1992; Calvert and Brown, 1986; Calvert *et al.*, 1982; Calvert *et al.*, 1997; Calvert *et al.*, 1996a; Calvert *et al.*, 1996b; Calvert *et al.*, 1998a; Calvert *et al.*, 1998b; Calvert *et al.*, 1996; Calvert *et al.*, 1997; Jacobs and Calvert, 1995), and O'Grady's group at the University of Guelph (Cory *et al.*, 1993; Minors and O'Grady, 1995; Minors and O'Grady, 1998; O'Brien *et al.*, 1992; O'Grady and Horne, 1995a; O'Grady and Horne, 1995b; O'Grady and Horne, 1995c; O'Grady and Horne, 1998).

Calvert published one of the earliest reports of Dobermann DCM in 1982, noting that Dobermanns accounted for 80% of all cases of DCM at the University of Georgia Veterinary Teaching Hospital. His observations of DCM in this breed included a low frequency of atrial fibrillation but a high frequency of ventricular arrhythmias compared with other breeds, a relative lack of signs of right heart failure, and a poor prognosis.

As has been frequently reported with other breeds, a strong male predisposition was noted in this early report. However, in two studies reported in 1997, the same group found equal number of males and females affected with DCM in one (Calvert *et al.*, 1997), and a marked male predisposition in the other (Calvert *et al.*, 1997). O'Grady and Horne did not find any sex predisposition in their study at a 2.5 year follow up (O'Grady and Horne, 1995b), although at a 4.5 year follow up, the prevalence of DCM or occult DCM in males was 78%, and 50% in females. These seemingly conflicting results can be explained by a tendency in most studies for both sexes to be equally affected with occult DCM, but for males to be more

affected with overt DCM, or to be affected at a younger age than females. Over 50% of dogs that present with overt congestive heart failure are between six and nine years of age, although the range is much wider (2 to 14 years) (Calvert *et al.*, 1997).

#### *2.2.1.1 Sudden death in Dobermanns*

From their earliest reports, Calvert's group (1986) noted a tendency to sudden death, and Calvert considered this a more common manifestation of DCM than overt congestive heart failure. He suggested that virtually all Dobermanns had occult arrhythmias prior to the onset of congestive heart failure (Calvert, 1986). A predisposition in Dobermanns towards sudden death had been previously noted in the literature, although this had not always been specifically linked to dilated cardiomyopathy in the reports (James and Drake, 1968; Taylor, 1983). James and Drake postulated that the sudden death in their series of eleven Dobermanns was associated with degenerative changes in the His bundle, which they believed resulted in a fatal form of bradyarrhythmia. The significance of the histological changes described is questionable. The authors were medical rather than veterinary pathologists, and they attached great importance to the presence of cartilage in the His region. Cartilage is a normal finding in the trigone region of canine hearts, although not of human hearts (Miller *et al.*, 1964). Although it is tempting to implicate DCM in this series, James and Drake were probably describing a heterogeneous group. One of their cases died during a series of convulsions after falling in sheep dip the day previously, and two were young puppies that died after being anaesthetised for ear-trimming.

Calvert and others (1997) compared histopathological changes in Dobermanns that had died suddenly with Dobermanns that had died of congestive heart failure, and found identical lesions in both groups. Both groups had multifocal areas of interstitial fibrosis, loss of myocytes with replacement by fibrous tissue or occasionally, adipose cells. Hazlett and others (1983) also reported myofibre degeneration with fatty replacement in Dobermanns with DCM, and Everett and others (1999) found adipose cell replacement in 30/32 Dobermanns with DCM examined at necropsy. Replacement of myocytes with fat cells has been reported in boxer cardiomyopathy, and is a feature shared with human arrhythmogenic right ventricular cardiomyopathy (Harpster, 1983; Thiene *et al.*, 1988; Wotton, 1998b), but has not been mentioned in most reports of the histopathological changes associated with canine DCM (Sandusky *et al.*, 1984; Van Vleet *et al.*, 1981). Arrhythmogenic right ventricular cardiomyopathy in people is associated with a tendency to sudden death as a result of ventricular arrhythmias, although, as in boxers, the source of these arrhythmias is usually the right ventricle (Fontaine *et al.*, 1977; Kraus *et al.*, 1999) rather than the left ventricle (as is suspected in Dobermanns). In their study of Dobermanns with DCM, Everett and others (1999) found changes exclusively in the left ventricular free wall, with up to 50% of the ventricular wall showing characteristic myofibre degeneration and fibrofatty replacement.

Calvert's group has documented progressive ventricular tachyarrhythmias in Dobermanns by 24 hour ambulatory (Holter) ECG monitoring, and shown that the presence of sustained ventricular tachycardia was associated with sudden death (Calvert, 1991e; Calvert, 1992; Calvert *et al.*, 1997). The same workers have used



signal averaged electrocardiography to evaluate suspected late potentials in Dobermanns with occult DCM, a technique that has been used in risk stratification in human ventricular arrhythmias (Martínez-Rubio *et al.*, 1995). Calvert's group has reported an absence of these signal averaged ECG abnormalities in normal Dobermanns (Calvert, 1991d; Calvert *et al.*, 1998a). Not all episodes of syncope in Dobermanns with DCM are associated with ventricular tachyarrhythmias; Calvert's group (1996) showed that sinus bradycardia and sinus arrest also occur, and may result in clinical signs.

#### *2.2.1.2 Occult DCM in Dobermanns*

Calvert (1986) described an "occult" stage, characterised by M-mode echocardiographic changes that preceded the onset of clinical signs. He reported M-mode values in three groups of Dobermanns: clinically normal Dobermanns without any physical, electrocardiographic or radiographic signs of DCM; asymptomatic Dobermanns with DCM, and Dobermanns with DCM and congestive heart failure. The definition of DCM was based on physical, radiographic, echocardiographic and post mortem findings. It is not clear from the report whether the asymptomatic dogs diagnosed with DCM demonstrated all these abnormalities, or just echocardiographic changes. However, all dogs designated as 'affected' subsequently died showing signs consistent with DCM.

O'Grady's group also addressed the issue of identifying Dobermanns with occult DCM by echocardiography (O'Grady and Horne, 1992; O'Grady and Horne, 1995b; O'Grady and Horne, 1995c). They adopted a slightly more stringent approach to the identification of occult DCM, and prospectively evaluated 193

asymptomatic Dobermanns by physical examination, a three-minute ECG, and two-dimensional guided M-mode and colour Doppler echocardiography. Of the original 193 Dobermanns, 103 were examined at least once subsequently (mean follow-up 28.3 months, with a range of 2-48 months). During this follow-up period, 28.2% of dogs followed developed DCM and congestive heart failure, or died suddenly (O'Grady and Horne, 1995b). They compared the original echocardiographic values of dogs that developed DCM with the original values of Dobermanns that remained asymptomatic with stable echocardiographic values for a minimum of two years (O'Grady and Horne, 1995a). They suggested echocardiographic predictors of DCM were a left ventricular diastolic dimension greater than 46mm, and a left ventricular systolic dimension greater than 38mm (O'Grady and Horne, 1995b).

These values are similar to those used by Calvert's group, namely, a left ventricular diastolic dimension of greater than 45mm in dogs weighing less than 38kg, and a diastolic dimension greater than 49mm in dogs over 37kg. However, the two groups differed in normal values for left ventricular fractional shortening, with Calvert's group asserting that values less than 25% were "unequivocally abnormal", although O'Grady and Horne showed a mean value of 21% in their "normal" Dobermanns, with a range of 13-30% (Calvert *et al.*, 1997; O'Grady and Horne, 1995b). Smucker and others (1990) evaluated left ventricular function by echocardiography and invasive haemodynamics in asymptomatic Dobermanns, and found evidence of reduced left ventricular ejection fractions compared with a group of crossbred dogs, but no evidence of elevated filling pressures, providing further evidence for the existence of an occult stage of DCM.

In terms of ventricular arrhythmias and a diagnosis of occult DCM, Calvert suggested that few normal Dobermanns have more than 50 ventricular premature complexes (VPC) over a 24 hour period by Holter monitoring, whereas all of a group of 42 Dobermanns with occult DCM had greater than 50 VPCs / 24 hours, ranging from 63 to over 35,000 (Calvert, 1991b). O'Grady and Horne found that all 15 dogs with VPCs on a three minute ECG went on to die of DCM within the follow-up period (O'Grady and Horne, 1995c). They calculated an overall incidence of occult and overt DCM of 44.7% in their group of 103 asymptomatic dogs, suggesting a very high prevalence in this sample. It is possible that sampling bias occurred if this group of dogs belonged to owners especially concerned over DCM, if these dogs were being screened because of related dogs known to be affected.

Calvert observed that seven male Dobermanns were imported into the US in the 1940s, which went on to have a major impact on the breed such that most Dobermanns in the US today trace back to these seven dogs. They were closely related, and three of them died of heart disease between the ages of seven and ten years (Calvert, 1986). With a familial basis for the disease in Dobermanns, it is easy to surmise how selective breeding might lead to such a high prevalence (Hammer *et al.*, 1996; Meurs, 1998).

### *2.2.1.3 Survival in Dobermanns with DCM*

The largest study of survival in symptomatic Dobermanns was a retrospective study by Calvert's group (1997), of 66 dogs with advanced congestive heart failure. Median survival was 6.5 weeks, with a range of 1 day to 60 weeks. These survival times do not represent much improvement on the median survival

time reported by Calvert in 1982 in 20 affected Dobermanns. No attempt was made to standardise treatment in the 1997 retrospective study, with all receiving frusemide, 26/66 dogs receiving ACE inhibitors, 5/66 receiving propranolol, and 8/66 receiving L-carnitine, amongst other therapies. Of the 66 dogs, two were euthanased for non-cardiac disease, and 30 dogs were euthanased for intractable or recurrent heart failure. The other 34 dogs died suddenly or of congestive heart failure. The eight week survival rate was 34%, and only 3% of dogs survived more than 12 months. Dogs with atrial fibrillation or signs of biventricular failure had a worse prognosis (mean survival 4.1 weeks, and 5.6 weeks respectively) than dogs with left heart failure and sinus rhythm (mean survival 11 weeks) (Jacobs and Calvert, 1995).

In one study of dogs with DCM, mean survival in 28 Dobermanns was only  $50 \pm 53$  (SD) days, compared with  $147 \pm 202$  days in 31 dogs of other breeds (Domanjko-Petric *et al.*, 1999). In another study of survival in 34 dogs with DCM (including 16 Dobermanns), 72% of the Dobermanns died in the first week compared with 23% of other breeds, and no Dobermann survived longer than five months (compared with 23% survival at five months in the other breeds of dog) (Lamontagne and DiFruschia, 1991). Comparing survival studies is more difficult than in human studies unless euthanased dogs are censored, because the timing of euthanasia may vary between clinicians. This is particularly true of euthanasia in the first week following diagnosis, as some clinicians may elect to euthanase Dobermanns rather than treat aggressively, knowing their tendency for poor survival times. This is less likely to be a factor in other breeds, where the specific survival times are less characterised, and the chances of long term survival are generally perceived to be better. In another study of survival in dogs with DCM, although Dobermanns were

the most commonly represented breed, breed was not an independent risk factor (Monnet *et al.*, 1995).

### 2.2.2 DCM in English Cocker spaniels

There have been fewer studies of DCM in English cocker spaniels than in Dobermanns. DCM is reported in American as well as English cocker spaniels, but there may be significant differences in the disease between the two breeds. In an analysis of the Purdue University VMDB, the prevalence of DCM in English cocker spaniels was 0.69% (compared with 0.34% in American cocker spaniels) (Sisson and Thomas, ). Out of 260 dogs with DCM seen at the University of California, Davis, none were English cocker spaniels, whereas American cocker spaniels made up 10% of cases (Kittleson, 1998). This suggests there may be a geographical variation, either in breed popularity or in disease prevalence. Certainly the incidence has been reported to be higher in specific breeding kennels (Staadén, 1981), and common ancestry amongst a group of affected dogs has been noted in another study (Thomas, 1987).

Staadén's study (1981) was one of the earliest reports describing cardiomyopathy in English cocker spaniels, although Detweiler had previously reported an increased incidence of congestive heart failure in male cocker spaniels (whether English or American was not specified) (Detweiler, 1964). Eleven affected English cocker spaniels were identified over a five-year period in Staadén's study centred on one kennel (Staadén, 1981). Three dogs were found dead, but these were found to have pulmonary oedema on post mortem, and may have suffered deaths due to congestive heart failure that were unobserved, rather than arrhythmic deaths as

seen in many Dobermanns. The other eight dogs all developed dyspnoea and signs of congestive heart failure. Cardiac murmurs were detected in five dogs, and all dogs were in sinus rhythm, with ventricular ectopic complexes noted in one. One dog had pulsus alternans. Tall amplitude R waves were noted on electrocardiography in all dogs evaluated by ECG, and in two other asymptomatic bitches in addition.

Post mortem findings included increased heart mass: bodyweight ratio, and mild thickening of the mitral and tricuspid valves. Histopathological examination showed foci of acute myocardial necrosis, and in one dog, foci of dense mature collagen. Evidence of chronic right heart failure was suspected from hepatic changes in four dogs.

The same kennel was the subject of further scrutiny by Gooding *et al.* (1982). Forty-nine dogs were examined, and divided into five groups based on electrocardiographic and radiographic findings (Gooding *et al.*, 1982). Group 1 dogs had normal radiographs and ECGs. Group 2 dogs had normal chest radiographs, but ECG evidence of left or biventricular hypertrophy and a normal mean electrical axis (MEA). Group 3 dogs had ECG and radiographic evidence of "left ventricular or biventricular hypertrophy". Group 4 dogs had a right axis deviation, and three dogs in Group 5 had left axis deviation.

Five "normal" dogs from Group 1, and five dogs from Groups 2, 3, 4 and 5 also underwent cardiac catheterisation and angiography. Three of these dogs were subsequently necropsied; two were normal control dogs from Group I, one of which was found to have a moderately thickened left ventricular free wall. The other dog was from Group 4 (right axis deviation), and had some localised septal hypertrophy

bulging into the left ventricular outflow tract. The results of two related animals that had been previously necropsied were also reported: one had generalised chamber dilation, and the other had concentric hypertrophy with focal areas of myocardial necrosis.

The authors suggested from these results that the cardiomyopathy in this kennel began as a form of hypertrophic cardiomyopathy, and progressed to a form of dilated cardiomyopathy. The evidence for the left ventricular hypertrophy described in the Group 2 dogs is based entirely on electrocardiographic evidence, yet the ECG criteria used did not distinguish between left ventricular hypertrophy and left ventricular dilation. No dog from Group 2 was necropsied to support these claims. Similarly, the radiographic criteria used did not distinguish between left ventricular hypertrophy and dilation. Although the dog from Group 4 and the related dog with concentric hypertrophy clearly showed no signs typical of dilated cardiomyopathy, these two dogs bear similarities with one of the normal control dogs, which was also said to demonstrate some left ventricular hypertrophy. The claims of left ventricular hypertrophy in the Group 2 dogs were not substantiated, let alone the evidence of a progression from left ventricular hypertrophy to left ventricular dilation. An increased heart : bodyweight ratio does not distinguish between eccentric hypertrophy (as seen in dilated cardiomyopathy) and concentric hypertrophy (as seen in hypertrophic cardiomyopathy).

The same authors were no longer making the same claim in a subsequent article, and noted that neither electrocardiography nor radiography is a sensitive technique for distinguishing hypertrophy from dilation (Gooding *et al.*, 1986b). They established normal left ventricular chamber dimensions using unguided M-



mode echocardiography in a group of asymptomatic cocker spaniels from the same kennel as in the previous study (Gooding *et al.*, 1982). Inclusion criteria included normal physical examination, normal thoracic radiographs and a lead II R wave amplitude less than 3.0 mV. They identified five animals that they considered to have borderline left ventricular function, on the basis of a reduced left ventricular fractional shortening ( $<27\%$ ), and excluded these dogs from the main group when establishing normal values for dimensions. The values obtained in this study were compared with the results from asymptomatic cocker spaniels believed to be affected with dilated cardiomyopathy on the basis of electrocardiographic or radiographic evidence of ventricular enlargement (Gooding *et al.*, 1986b). Three of the dogs had a history of a dyspnoeic episode consistent with left heart failure, although were clinically normal at the time of examination. The specific echocardiographic findings are discussed further in chapter 3, but the affected dogs all showed evidence of increased left ventricular systolic dimensions, with similar septal thickness measurements to the normal group. There was no evidence of concentric hypertrophy, and no further mention of hypertrophic cardiomyopathy.

No evidence was found of left ventricular hypertrophy in a British report of a series of eight cases of dilated cardiomyopathy in English cocker spaniels (Thomas, 1987). Post mortem findings were available in five of these dogs, four of which had biventricular dilation, and one had right ventricular dilation with normal left ventricular dimensions. Mild endocardiosis lesions were found in four dogs, but it was not felt the changes were sufficiently severe to account for the other changes. The dogs in the study by Thomas shared some features with the dogs described by Gooding and coworkers (predominant left heart failure, increased R wave amplitudes



in lead II, deep Q waves in limb leads, and deep S waves in chest leads CV<sub>6</sub>LU and CV<sub>6</sub>LL). He noted a marked male predisposition (7/8 dogs were male). Gooding's reports did not mention gender. While Gooding suggested that affected dogs were more likely to be older, some of the dogs in Thomas's study were comparatively young (range: 10 months to 8 years, with a mean age of 3 years).

#### *2.2.2.1 Survival in cocker spaniels*

The cocker spaniels studied by Staaden and Gooding's group appeared to have had a relatively benign clinical course. Several dogs had a history of surviving episodes of congestive heart failure, and remained in "spontaneous remission" without medication. In the study by Thomas, three out of eight dogs died within the first month following diagnosis, but the remaining dogs were either euthanased with non-cardiac disease or were still alive at the time of writing the report, in one case, up to two and a half years later (Thomas, 1987). Wotton also commented on the long survival periods in cocker spaniels (Wotton, 1998a).

#### *2.2.2.2 Comparison with American cocker spaniels*

Both English and American cocker spaniels are descended from the Field Spaniel. However, there appear to be some differences in the DCM seen in the two breeds. Low plasma taurine levels have been reported in American cocker spaniels with DCM (Gavaghan and Kittleson, 1997; Kittleson *et al.*, 1997; Kramer *et al.*, 1995). In a multicentre study examining taurine levels in dogs with DCM, there was no significant difference between levels in the control and DCM dogs, although a

subset of DCM dogs had very low levels (Kramer *et al.*, 1995). Seven out of thirteen dogs with low plasma taurine levels were American cocker spaniels, some with plasma taurine levels less than 25 nmol/mL. In an unpublished study by Darke, Kittleson and the author, evaluating taurine levels in English cocker spaniels using the same assay as reported by Kittleson (Kittleson *et al.*, 1997) no evidence was found of taurine deficiency.

## 2.3 STUDY ANIMALS

### 2.3.1 Selection criteria

Normal dogs were selected from privately owned German short-haired pointers. All dogs were clinically healthy and were normal on physical examination, including auscultation.

The dogs with dilated cardiomyopathy were all cases seen at the Small Animal Clinic at the Royal (Dick) School of Veterinary Studies, University of Edinburgh. Some dogs were seen as first opinion cases, but the majority were cases referred by private veterinary surgeons. The study was restricted to Dobermanns or English cocker spaniels diagnosed with dilated cardiomyopathy.

The three main inclusion criteria for dogs affected with DCM were: (1) presence of signs of congestive heart failure; (2) a dilated hypocontractile heart (left ventricular fractional shortening < 20%); and (3) an absence of congenital, valvular or pericardial abnormalities. Dogs with minor valvular changes consistent with mild endocardiosis were not excluded.

The severity of heart failure on presentation was assessed using a modification of the New York Heart Association classification (Appendix 1) (The Criteria Committee of the New York Heart Association, 1973). Body surface area (BSA) was obtained from a table based on calculations using the following formula (Price and Frazier, 1998):

Formula 2.1 :

$$BSA = \frac{[10.1 \times \text{bodyweight (g)}]^{2/3}}{10\,000}$$

## 2.4 STATISTICAL METHODS

All values are expressed as mean  $\pm$  SD. The values were compared between GSH pointers, Dobermanns and cocker spaniels by one way analysis of variance when data were normally distributed, and by Kruskal-Wallis one way analysis of variance when a test for normality failed. When a significant difference was found between groups, multiple pairwise comparisons were made using a Tukey test for normally distributed data, or Dunn's method following a Kruskal-Wallis test. When two groups showed no statistical difference at a significance level of  $p < 0.05$  in pairwise comparisons, they were given the same superscript letter (graphs 2.1 – 2.3). Comparisons between the Dobermanns and cocker spaniels were made using an unpaired t-test when data were normally distributed with equal variances, otherwise they were compared by a Mann-Whitney rank sum test. Differences in proportions were calculated using a two-tailed Fisher exact test. All statistical analyses were carried out using a proprietary statistical software programme (SigmaStat 2.0, Jandel Scientific, San Rafael, CA, USA). Graphs were plotted using SigmaPlot (Jandel Scientific, San Rafael, CA, USA).

## 2.5 RESULTS

All the dogs with DCM had developed congestive cardiac failure prior to the studies. The therapy received prior to echocardiography varied, but all had received frusemide. Some dogs had also been treated with digoxin, angiotensin converting enzyme inhibitors, glyceryl trinitrate paste, and etamiphylline. Following echocardiography, all dogs were treated with frusemide 1-5mg/kg every 6-24 hours to effect, enalapril 0.5 mg/kg every 12-24 hours, and digoxin 0.11mg/m<sup>2</sup> daily. In addition, some dogs were randomised to receive pimobendan (an inodilator), or placebo. The clinical details of each case are listed in tables 2.2 and 2.3.

### 2.5.1 Normal dogs

The normal dogs comprised 13 German shorthaired pointers. Their mean age was  $5.5 \pm 3.3$  years, with a range of 2 to 12 years, and mean weight was  $26.8 \pm 3.8$  kg (range 22 - 34 kg). Nine of the dogs (1, 2, 3, 6, 7, 9, 10, 11 & 13) were working dogs, and had undergone varying degrees of physical training. The clinical details of each dog are shown in Table 2.1, together with resting heart rates. The cardiac rhythm in all dogs was sinus arrhythmia (which was confirmed by ECG recorded during echocardiography).

Table 2.1 Clinical details of normal German shorthaired pointers.

No.	sex	Age (years)	Weight (kg)	BSA (m <sup>2</sup> )	HR (bpm)
1	F	5	26	0.88	69.7
2	F	7	27	0.9	54.9
3	M	3	32	1.01	66.9
4	M	9	32	1.01	80.6
5	FN	2	26	0.88	89.7
6	F	2	22	0.78	109.8
7	F	2	24	0.83	98.9
8	FN	12	25	0.85	104.1
9	FN	9	29	0.94	95.4
10	M	6	34	1.05	67.9
11	F	8	23	0.81	79.4
12	M	2	23	0.81	86.3
13	M	5	26	0.88	83.3
<b>mean</b>		5.5	26.9	0.89	83.6
<b>SD</b>		3.3	3.8	0.09	16.1

BSA: body surface area, M: male, MN: neutered male, F: female, FN: neutered female.

## 2.5.2 Dobermanns with dilated cardiomyopathy

The 11 Dobermanns had a mean age of 8.3 years (SD  $\pm$  1.6 years), with a range of 6 to 11 years. Their mean weight was 37.5 (SD  $\pm$  7.8) kg, (range 30 - 46 kg). Only two females were represented, with the rest of the Dobermanns being entire or castrated males. The mean heart rate was 135.3 (SD  $\pm$  27.2) bpm, (range 89.5 - 187 bpm). Three dogs were in atrial fibrillation, and ventricular premature complexes were observed frequently during echocardiography in four dogs. Two Dobermanns had small pericardial effusions (dogs 9 and 10), but no evidence of cardiac tamponade. This fluid appeared to be a manifestation of congestive failure rather than a cause, as there was generalised chamber dilation with signs of severe left-sided congestive failure in these dogs.

The median survival time was 98 days (range 16-508 days), with only one dog dying a (probable) non-cardiac death (Dobermann 1). This dog developed

immune-mediated haemolytic anaemia and acute pancreatitis. She also developed a recurrence of congestive cardiac failure signs in the week prior to her death, so that it was difficult to isolate the principal cause of death. All the other Dobermanns died of causes related to their cardiac disease, with three dogs dying suddenly, three dogs dying with signs related to congestive heart failure, and three dogs being euthanased because of intractable cardiac failure. Deaths were classified as sudden if they were not preceded by a worsening of congestive signs.

Table 2.2 Clinical details of Dobermanns with DCM

Dog	sex	Age (years)	Weight (kg)	BSA (m <sup>2</sup> )	HR (bpm)	rhythm	NYHA class	Pim	Survival (days)	End-point
1	FN	8	32.5	1.02	137.0	SR	2	+	429	Non-cardiac death
2	M	7	30.0	0.96	172.7	SR / VPCs	2	-	150	Sudden death
3	FN	8	30.0	0.96	134.1	SR	2	-	181	Sudden death
4	M	6	53.0	1.40	133.7	SR	3	-	21	Euth with CHF
5	M	9	46.0	1.28	159.0	SR	2	-	61	CHF
6	M	9	37.5	1.12	89.5	SR /VPCs	2	-	88	Euth with CHF
7	MN	8	33.0	1.03	151.0	SR	2	+	381	Sudden death
8	M	9	34.5	1.06	105.6	SR /VPCs	2	+	508	Euth with CHF
9	M	10	30.5	0.98	163.9	AF	4	-	16	Euth with CHF
10	M	11	46.0	1.28	187.0	AF	3	-	81	CHF
11	M	6	40	1.17	130.2	AF /VPCs	3	-	98	CHF
mean	-	8.27	37.55	1.11	142.15	-	2.45	-	183.1	-
SD	-	1.56	7.79	0.15	28.62	-	0.69	-	173.8	-

BSA: body surface area, NYHA: New York Heart Association heart failure classification, Pim: whether pimobendan was added to therapy with frusemide, digoxin and enalapril, Euth: euthanased, CHF: congestive heart failure, M: male, MN: neutered male, F: female, FN: neutered female, VPCs: ventricular premature complexes, SD: standard deviation.

### 2.5.3 English cocker spaniels with dilated cardiomyopathy

The 11 cocker spaniels had a mean age of 6.3 years (SD  $\pm$  3.8 years), with a range of 2 to 15 years. Their mean weight was 14.7 (SD  $\pm$  1.3) kg, with a range of 12 to 16.5 kg. Only two were female, and all nine others were entire males. The mean heart rate was 140 (SD  $\pm$  31) bpm, with a range of 95 to 180 bpm. All the dogs were in sinus rhythm, although five dogs had pulsus alternans (figure 5.1). This has been reported before in cocker spaniels with DCM (Staadén, 1981).

Table 2.3 Clinical details of cocker spaniels with DCM

Dog	sex	Age (years)	Weight (kg)	BSA (m <sup>2</sup> )	HR (bpm)	rhythm	NYHA class	Pim	Survival (days)	End-point
1	M	4.5	14.5	0.59	167	SR / PA	3	-	150	Non-cardiac death
2	M	3.0	12.0	0.52	177	SR / PA	3	-	>1388	Alive
3	M	2.0	16.0	0.63	180	SR	3	+	51	Sudden death
4	M	5.0	15.0	0.60	170	SR	2	-	557	Non-cardiac death
5	M	10.0	16.5	0.64	157	SR / PA	2	+	995	Non-cardiac death
6	M	8.0	16.0	0.71	110	SR	2	+	>1084	Alive
7	M	4.0	15.0	0.60	141	SR / PA	3	-	>737	Alive
8	M	15.0	13.5	0.56	106	SR/APCs	2	+	396	Non-cardiac death
9	FN	8.0	14.0	0.58	113	SR / PA	2	+	>413	Alive
10	FN	7.0	14.0	0.58	123	SR	2	-	>373	Alive
11	M	3.0	15.0	0.60	95	SR	3	-	>512	Alive
mean	-	6.32	14.68	0.6	139.91	-	2.45	-	605.1	-
stdev	-	3.82	1.29	0.05	31.60	-	0.52	-	408.8	-

BSA: body surface area, HR: heart rate, NYHA: New York Heart Association heart failure classification, Pim: whether pimobendan was added to therapy with frusemide, digoxin and enalapril, M: male, F: female, FN: neutered female, APCs: atrial premature complexes, SR: sinus rhythm, PA: pulsus alternans

Median survival time was 512 days (range 51 to >1388 days). At the time of writing, six dogs were still alive with stable cardiac disease, four dogs had died or been euthanased because of non-cardiac disease (complications of diabetes mellitus, haemorrhagic gastroenteritis, nasal neoplasia, spinal disease), and one dog died suddenly within two months of diagnosis (dog 3). None of the cocker spaniels developed a second episode of congestive heart failure.

Two cocker spaniels did have some thickening of the mitral valve leaflets (dogs 8 and 9), but severe myocardial failure secondary to mitral regurgitation is uncommon in this breed, and these two dogs were felt to have clinical features such as pulsus alternans that were more suggestive of DCM.

#### 2.5.4 Comparison of Breed Groups

There were significant differences in bodyweight ( $p < 0.001$ ), with a Dunn's test showing significant differences in pairwise comparisons between the three groups (Graph 2.1). A one-way analysis of variance showed no significant difference in ages (Graph 2.2).

An analysis of variance on ranks showed significantly higher heart rates in the Dobermanns (median heart rate 137 bpm) and cocker spaniels (median 141 bpm) compared with the normal dogs (median 83 bpm) at a level of  $p < 0.001$  (Graph 2.3). Between the two groups with DCM, a Mann-Whitney test demonstrated no significant difference in NYHA class on presentation (Graph 2.4).

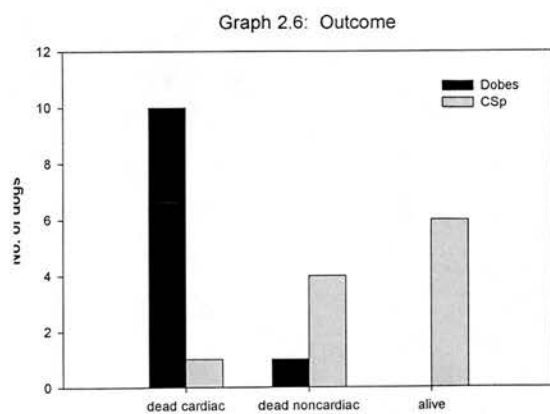
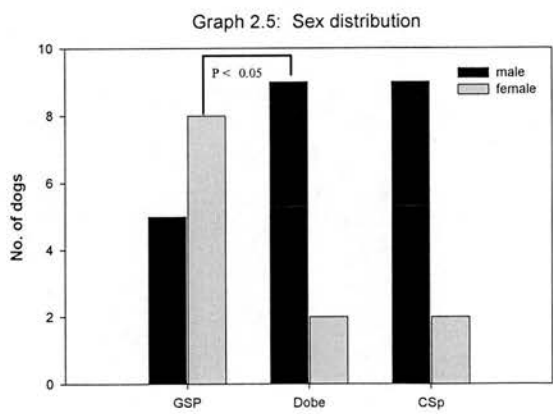
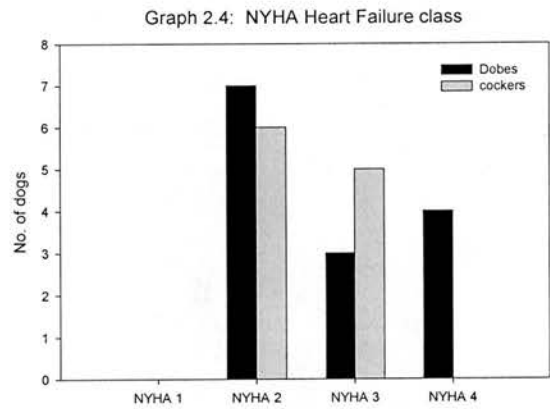
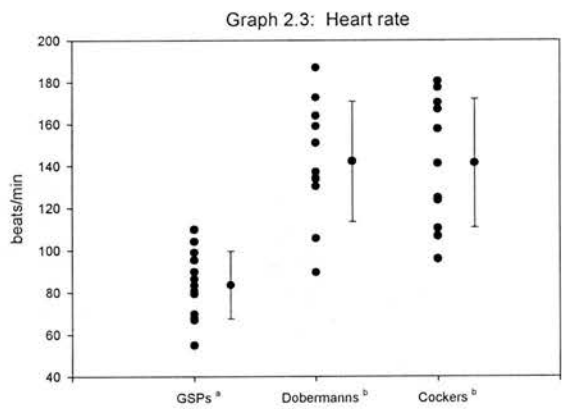
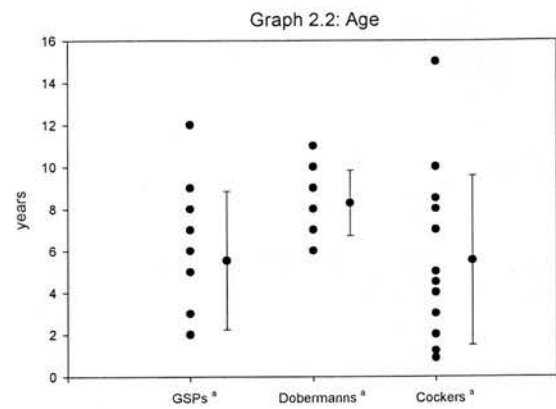
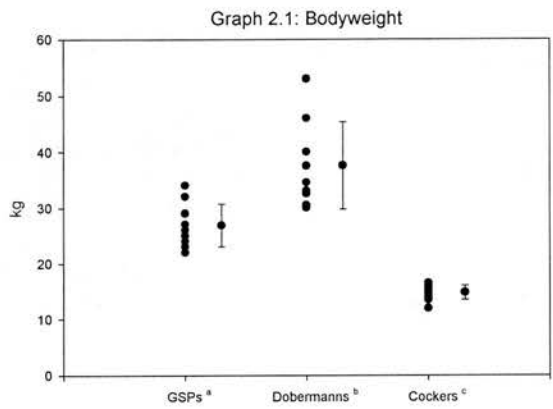


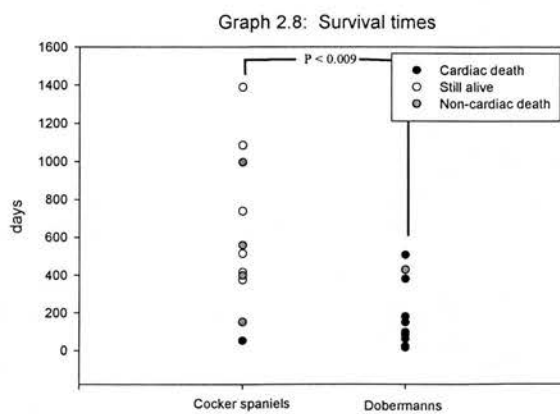
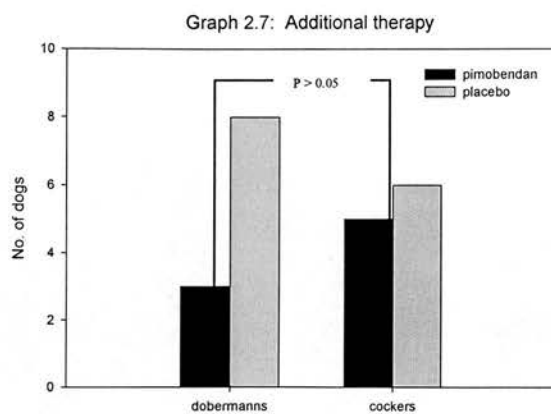
Both DCM breed groups comprised predominantly male dogs, with only two out of eleven dogs in each group being female (Graph 2.5). Although there was an identical sex distribution in the affected dogs, the proportion of males and females was significantly different from the normal dogs ( $p < 0.05$ ) when Dobermanns were compared with GSPs using a Fisher exact test, with eight normal females and five normal males.

Of the cocker spaniels that had died, only one out of five had died a cardiac-related death, and signs of congestive heart failure were well-controlled in all four of the other dogs at their time of death, with none of the dogs showing any signs of cardiac decompensation. In contrast, all but one of the Dobermanns died cardiac-related deaths, with seven dogs developing a recurrence of congestive heart failure (Graph 2.6). A Fisher exact test showed no difference in the proportion of dogs receiving pimobendan between the cockers and the Dobermanns (Graph 2.7).

There was a significant difference in survival ( $p < 0.009$ ) between the Dobermanns (median survival 98 days) and cockers (median survival 512 days). At a median follow-up time of 1084 days, six of the eleven cocker spaniels were still alive (range 373-1198 days), whereas all of the Dobermanns had died by 508 days (Graph 2.8).

Clinical details of animals





Groups with the same superscript letters were not significantly different in pairwise comparisons.

## 2.6 DISCUSSION

### 2.6.1 Choice of breeds with DCM

There were a number of reasons why Dobermanns and cocker spaniels were chosen as the study breeds. Not only were Dobermanns and cocker spaniels the two breeds most commonly seen with DCM at the R(D)SVS, but they also showed marked divergence in the clinical course of the disease. Furthermore, both breeds generally remain in sinus rhythm, even with severe dilated cardiomyopathy (in contrast with giant breeds affected with DCM). This facilitates assessment of many echocardiographic variables, and transmitral flow measurements in particular. Despite this, three of the Dobermanns were in atrial fibrillation. They were not excluded, despite the difficulties in data analysis, because of the small sample numbers. It was anticipated that there would be difficulties in recruiting sufficient cases for this type of study, even if the most commonly affected breeds were used. The low numbers were a severe limitation for survival analysis, because there were insufficient cases to be able to calculate proportional hazards analysis on any echocardiographic variables, after allowing for differences in breed, age and sex. Small group size is a common problem in clinical studies, but the consistent clinical course of DCM in the two respective breeds helped to minimise some of the survival variation that occurs in mixed population of dogs (Monnet *et al.*, 1995).

### 2.6.2 Choice of normal control group

The ideal choice for controls would have been normal Dobermanns and cocker spaniels. This would have eliminated much of the need for indexing variables, as each DCM group would have had directly comparable control animals.

Unfortunately it is extremely difficult to prove conclusively that an animal from a predisposed breed is definitively normal. There is no age by which it can be assumed a dog will not develop DCM, as the onset of DCM may be very late in some animals. One alternative was to use a group of mixed breed dogs, which are known to have a low prevalence of DCM. The heterogeneity of such a group would complicate the interpretation of indexed variables, and proved difficult logistically to organise for this study. Another alternative was to use a breed unlikely to develop DCM but with physical characteristics intermediate between the two affected breeds. German short-haired pointers met these criteria, and had the advantage of accessibility. They were also sufficiently short-coated that clipping was unnecessary, which facilitated owner compliance. One disadvantage was that many of the individuals in the final group were athletic, physically fit animals that might not be typical of most normal populations of pet dogs. However, this also provided some reassurance that these dogs did not have occult myocardial disease.

# CHAPTER 3: EVALUATION OF VENTRICULAR FUNCTION IN NORMAL DOGS AND DOGS WITH DCM USING M-MODE ECHOCARDIOGRAPHY

M-mode echocardiography is often used as the definitive technique for diagnosing dilated cardiomyopathy (DCM) in dogs (Keene, 1989). For this reason, it is important that reliable M-mode variables are chosen for distinguishing normal dogs from dogs affected with DCM. Most reports of ventricular function in canine DCM have concentrated on M-mode-derived variables, although little attention has been paid to comparison of M-mode values in different breeds affected with DCM. A number of M-mode variables have been indicated as having prognostic value in human DCM (Baker *et al.*, 1986; Benjamin *et al.*, 1981; Werner *et al.*, 1993), and it was hypothesised that there might be differences in some M-mode indices that would relate to the difference in survival times between the Dobermanns and English cocker spaniels in this study.

## 3.1 AIMS

1. To measure M-mode echocardiographic values in a group of normal German short-haired pointers, and to establish the repeatability of these measures.
2. To compare the M-mode echocardiographic results obtained in the normal dogs with those from a group of Dobermanns with DCM, and a group of English cocker spaniels with DCM.

3. To compare the results obtained in the two groups of dogs with DCM, to identify any differences in chamber dimensions or systolic function that might merit further investigation as prognostic indicators in canine DCM.

## 3.2 BACKGROUND

### 3.2.1 M-mode echocardiographic indices in human DCM

The primary features recognised in dilated cardiomyopathy are ventricular dilation, with a reduction in the extent of myocardial shortening (Corya *et al.*, 1974; Hayakawa *et al.*, 1984; McDonald *et al.*, 1972; Shah, 1988). Although LV mass is generally increased, wall thicknesses may be normal, or even reduced (Benjamin *et al.*, 1981).

Additional changes reported in DCM include reduced amplitude of excursion of septal and free wall motion (Corya *et al.*, 1974), and increased E-point to septal separation (EPSS) (Massie *et al.*, 1977). In one study of EPSS in human patients with DCM, there was no correlation with fractional shortening, but a good correlation with LV size (Pollick *et al.*, 1982). However, all the patients studied in this group had depressed FS% as well as increased EPSS, and the association between EPSS and systolic function has been consistently demonstrated in studies including patients with a wider spectrum of LV function (Child *et al.*, 1981; Engle *et al.*, 1983; Massie *et al.*, 1977).

Gardin and others noted a greater degree of early aortic valve partial closure in patients with DCM than in patients with aortic insufficiency or in normal subjects (Gardin *et al.*, 1984b).

When screening relatives of DCM patients by M-mode echocardiography, identification of affected subjects is often based on increased LV end-diastolic diameter (exceeding 95% confidence limits of published normal values) and/ or a FS% <25% (Baig *et al.*, 1998; Lestuzzi *et al.*, 1991).

### 3.2.2 M-mode prognostic indicators in human DCM

Although increased LV diameter is a widely found abnormality in human DCM, a number of studies have failed to associate it with any prognostic value (Keogh *et al.*, 1988; Pelliccia *et al.*, 1994). A subset of patients with only mild LV dilation do not appear to have an improved prognosis, and may represent a heterogeneous group consisting of early DCM and severe LV dysfunction (Gavazzi *et al.*, 1993; Keren *et al.*, 1990). It is possible that the sample selection may influence the effect of LV dilation on prognosis, as one study of minimally symptomatic patients with DCM did identify increased LV systolic dimensions as an indicator of poorer outcome (Nagaoka *et al.*, 1997).

Interestingly, despite the apparent lack of usefulness of linear minor axis dimension measurements, a number of studies have shown the prognostic value of LV volumes calculated by 2D echocardiography (Diaz *et al.*, 1987; Juilliere *et al.*, 1988; Romeo *et al.*, 1989). This discrepancy might be explained by a genuine association between prognosis and LV volumes, but poor correlation between LV diameter and volume. This is indeed likely to be the case, as is discussed in the subsequent section on 2DE volume calculations. Alternatively, the better prognostic value of LV volumes might reflect an association between poor outcome and increased volume without an equivalent increase in LV diameter. However, this



does not appear to be true, as an increase in diameter relative to long axis length appears to be a poor prognostic sign (Douglas *et al.*, 1989).

Although LV mass is invariably increased in DCM, the degree of LV hypertrophy has been reported to be a useful prognostic factor, with increased wall thickness relative to cavity dimensions being associated with improved outcome (Benjamin *et al.*, 1981; Shah, 1988).

Depressed FS% has been reported to have prognostic value in several studies (Baker *et al.*, 1986; Nagaoka *et al.*, 1997; Werner *et al.*, 1993), though not in all (Douglas *et al.*, 1989; Keogh *et al.*, 1988). One study showed an improved outcome in children with DCM who demonstrated an increase in FS% over time (Lewis, 1994). Velocity of circumferential fibre shortening has also proved useful as a prognostic indicator (Baker *et al.*, 1986; Werner *et al.*, 1993).

### 3.2.3 M-mode assessment of canine DCM

From the early 1980s, M-mode echocardiography became an essential part of the evaluation of ventricular function in dogs with DCM, and has since become well-established as the principal modality for diagnosis. M-mode echocardiography has been used to identify both chamber dilation and abnormal systolic function (Gooding *et al.*, 1986b; Kittleson *et al.*, 1985; Koch *et al.*, 1995; Lombard, 1984a; Tidholm and Jonsson, 1996; Vollmar, 1999). Accurate identification of LV dilation depends on the availability of breed-specific normal reference values, and several studies have reported M-mode values in normal dogs along with values in affected dogs (Calvert and Brown, 1986; Dukes McEwan, 1998a; Vollmar, 1999). Accurate identification of dogs affected with DCM is still problematic when the diagnosis is

based on echocardiographic criteria alone in asymptomatic animals, unless those dogs are shown subsequently to progress to overt congestive heart failure.

Most reports of M-mode echocardiography in dogs with DCM have focussed on the decrease in FS%, and to a lesser extent, the increase in left ventricular diameter (Kittleson *et al.*, 1985; Koch *et al.*, 1995; Monnet *et al.*, 1995). Where the lower limits of normality lie for FS% is a matter of controversy, varying anywhere from <18% (Dukes McEwan, 1998a) to <33.7% (Boon, 1998b), yet Vollmar reported a mean FS% as high as  $20.7 \pm 8.3\%$  in Irish Wolfhounds with advanced DCM and signs of congestive heart failure.

EPSS (Bonagura and Herring, 1985; Calvert and Brown, 1986; Koch *et al.*, 1995), Vcf (Calvert and Brown, 1986; Kittleson *et al.*, 1985), PWth% (Calvert and Brown, 1986; Kittleson *et al.*, 1987; Lombard, 1984a; Smucker *et al.*, 1990) and ESV index (Kittleson *et al.*, 1984; Kittleson *et al.*, 1985; Ware *et al.*, 1990) have all been reported as indices of abnormal ventricular function in dogs.

Qualitative M-mode changes reported in canine DCM include a “B” shoulder between the A and C points of mitral valve closure, said to be associated with increased LV end-diastolic pressures (Bonagura and Herring, 1985; Lombard, 1984a).

The only M-mode variable reported in the literature to have prognostic value is left ventricular free wall thickening (Monnet *et al.*, 1995). In one of the few studies of prognostic indicators in canine DCM, free wall thickening was not found to be an independent predictor of outcome, but its addition provided additional strength in multivariate Cox stepwise regression analysis.

### *3.2.3.1 M-mode echocardiography in Dobermann DCM*

Dobermanns with DCM have received particular echocardiographic attention compared with other breeds. Calvert's study in 1982 was one of the earliest reports of the use of M-mode echocardiography in canine DCM (Calvert *et al.*, 1982). Calvert and Brown followed this with further studies in 1986 (Calvert and Brown, 1986), and 7/12 dogs in Lombard's study (1984) were Dobermanns (Lombard, 1984a).

O'Grady and coworkers in Ontario have used M-mode echocardiography to follow asymptomatic Dobermanns prospectively in an attempt to establish echocardiographic markers of occult DCM in this breed (O'Grady and Horne, 1992; O'Grady and Horne, 1995a; O'Grady and Horne, 1995b; O'Grady and Horne, 1995c). O'Grady and Horne performed serial 2D-guided M-mode studies over a period of two years, excluding values obtained from dogs that demonstrated progressive ventricular enlargement, showed deterioration in FS%, had evidence of ventricular ectopy, or developed signs of DCM during the study period. Fifty-one out of 192 Dobermanns met these criteria, and were classified as "normal", in that they failed to show signs of deterioration over a two year follow-up period. These dogs has a mean FS% of 21%, mean LVDd of 3.91cm, and a mean LVDs of 3.1cm.

Minors and O'Grady (1998) reported the results in detail of 29 apparently healthy Dobermanns, six of which developed occult DCM over a period of up to a year (Minors and O'Grady, 1998). Twelve other Dobermanns were classified as 'occult' DCM based on their initial echocardiogram. They found significant differences between normal and occult DCM groups for LV diameter (in systole and

diastole), FS%, and for the ratio of systolic wall thickness to systolic LV diameter. However, it should be noted that the criteria for inclusion in the occult DCM group in the first place were based on increased LV diameter and reduced FS%, so it is not surprising that there were significant differences between the groups based on these variables. They repeated echocardiography during an infusion of dobutamine at 5µg/kg/min, but found no additional discriminatory power with the dobutamine stress test.

In the 32 Dobermanns examined by Sottiaux and Amberger, the reported mean FS% was considerably higher than Minor's and O'Grady's value ( $33.8 \pm 7.0\%$ , versus  $21.7 \pm 0.76\%$ ). The latter workers' value for SD is surprisingly small, if correct. Calvert published values for FS% of  $34.2 \pm 1.81$  in clinically normal Dobermanns, versus  $18.3 \pm 5.91$  in Dobermanns with DCM (Calvert and Brown, 1986). In a subsequent study of occult DCM in Dobermanns, Calvert performed serial echocardiography on asymptomatic Dobermanns predominantly submitted for screening purposes (Calvert *et al.*, 1997). Those dogs that subsequently developed congestive heart failure had more abnormal values at the onset of the study than the Dobermanns that went on to die suddenly. At the onset of congestive failure, he reported the mean FS% as  $<15\%$ , mean LVDd  $>5.8\text{cm}$ , mean EPSS  $>1.6\text{cm}$ , and mean Vcf  $<0.84\text{ circ/s}$ , although he did not actually give the values at first presentation. He did state, however, that M-mode variables considered "unequivocally abnormal" include FS%  $<25\%$ ; LVDd  $>45\text{mm}$  in dogs weighing  $<38\text{kg}$  and  $>49\text{mm}$  in dogs weighing  $>37\text{kg}$ ; EPSS  $>8\text{mm}$ , and Vcf  $<1.5\text{ circ/s}$ .

Smucker and co-workers found subnormal systolic function in asymptomatic Dobermanns compared with mongrels, using both %PWth and angiographic ejection fractions (Smucker *et al.*, 1990).

#### *3.2.3.2 M-mode echocardiography in English cocker spaniels*

English cocker spaniels with DCM or presumed occult DCM have also been the subject of echocardiographic studies (Gooding *et al.*, 1986b). Gooding, Robinson and Mews studied a kennel of cocker spaniels with a high incidence of congestive heart failure, and found that nearly half the apparently healthy cocker spaniels in this group had LVDs dimensions and LVPWd which exceeded the 95% confidence intervals published in 1983 by Boon and others (Gooding *et al.*, 1986a). Five out of 17 of these asymptomatic dogs had FS% values less than 27%. Although an earlier report by the same authors had suggested that the cause of heart failure in these dogs was a cardiomyopathy characterised by concentric hypertrophy (Gooding *et al.*, 1982), a later echocardiographic report suggested that they were actually affected with dilatation (dilated) cardiomyopathy (Gooding *et al.*, 1986b). It has been suggested that English cockers with DCM have less severe impairment of systolic function than found in other breeds (Fox, 1988)

### 3.3 MATERIALS AND METHODS

#### 3.3.1 Equipment

All M-mode echocardiographic studies were carried out using a Vingmed CFM 700 ultrasound system (Vingmed CFM 700, Dasonics, Sonotron, Bedford, UK) using either a 5.0 MHz or 2.25 MHz annular array transducer. The transducers were mechanically steered with dynamic focussing. The 5.0 MHz probe produced 2D images with a central bandwidth around 5.0 MHz and the 2.25 MHz probe had a central bandwidth around 2.25MHz. A simultaneous ECG was recorded in all studies (limb lead II) using pre-gelled adhesive electrodes attached to the main pads of the feet, or to the limbs above the carpus and tarsus (F60, Skintact ECG pads, HA West Ltd, 41 Watson Crescent, Edinburgh, UK ). Electrodes were further secured using permeable non-woven synthetic adhesive tape (Micropore, 3M Health Care Ltd, 3M House, Morley St, Loughborough, Leics, UK). Studies were recorded onto VHS video tape by a Panasonic AG 6200 video cassette recorder. Still transparencies could be recorded onto 35 mm film using a freeze frame recorder (Polaroid UK Limited, Ashley Road, St Albans, Herts, UK).

##### *3.3.1.1 Echocardiograph settings*

The depth setting was adjusted to maximise the size of the cardiac image within the sector. The required depth would vary between 10 to 12 cm for the German shorthaired pointers, 8 to 12 cm for the cocker spaniels, and 10 to 14 cm for the Dobermanns. The sector angle was generally 90°, which yielded a frame rate of 26 frames/second at these depth settings during 2D echocardiography. Post-processing controls were pre-set, so that reject and compression levels were the same

for each study. The time gain compensation controls were adjusted as an even 'ramp' up to the maximum image depth. Overall gain settings were adjusted for each animal to produce the optimal image quality. The M-mode horizontal sweep speed was set at 2 seconds, which was the maximum sweep speed available. No other setting changes were usually required from the 2D settings. M-mode and 2D imaging was generally carried out using the 5 MHz transducer.

### 3.3.2 Methods

Dogs were scanned unsedated, and manually restrained in right lateral recumbency on a purpose-built table. The table consisted of a metal base with a removable rigid perspex top, with had a V-shaped section removed halfway along the edge of one of the long sides. This was to facilitate access for the transducer to the dependent part of the thorax, so that right parasternal views were obtained with the dog lying in right lateral recumbency.

M-mode images were derived from a 2D right parasternal short axis image, making every effort to obtain a true short axis cross-section by selecting images with a circular ventricular cavity. M-mode studies of the left ventricle were made at the chordal level, in agreement with ASE recommendations (Sahn *et al.*, 1978). The cursor was placed so that it bisected the left ventricle, which frequently necessitated moving the image over to one side of the screen to avoid traversing chordae or papillary muscles. M-mode studies were also obtained at the mitral valve level, and at the level of the aorta and left atrium. For the aortic valve level, attempts were made to image two aortic valve leaflets whilst placing the cursor as close to the body

of the left atrium as possible, although frequently the cursor passed instead through the left auricular appendage.

### 3.3.3 Protocol

#### *3.3.3.1 Normal GSH pointers, reproducibility studies*

All dogs were clinically normal on physical examination, and had no history that would suggest cardiac disease. The full details of these dogs are described in chapter 2. Eleven dogs were scanned on one occasion only, and two dogs were scanned on each of five successive days.

#### *3.3.3.2 Dogs with DCM*

All dogs had been presented to the R(D)SVS for diagnosis and/or treatment of dilated cardiomyopathy. A diagnosis of DCM was based on the presence of congestive heart failure with a dilated hypocontractile heart and an absence of congenital, valvular or pericardial abnormalities. The selection criteria are described in more detail in Chapter 2.

### 3.3.4 M-mode measurements

Measurements were made off-line from the recorded video cassette tapes. The Vingmed system integral measuring software was accessed by entering a calibration code recorded in the lower left corner of the recorded image when prompted. The measurement menu available for M-mode image analysis allowed



measurement of LV, left atrial and aortic dimensions in diastole and systole using electronic calipers positioned by means of a tracker ball.

The following variables were measured: left ventricular diameter in diastole (LVDd) and systole (LVDs); interventricular septal thickness in diastole (IVSd) and systole (IVSs); left ventricular posterior wall thickness in diastole (LVPWd) and systole (LVPWs); mitral valve E point to septal separation (EPSS); and aortic (Ao) diameter and left atrial (LA) diameter. The timing of measurements followed ASE recommendations (Sahn *et al.*, 1978), so that end-diastole was taken from the start of the QRS complex, and end-systole was taken at the nadir of septal motion. At the aortic valve level, systole was taken at maximum aortic excursion. Measurements were made from “leading edge to leading edge”, so that when measuring left ventricular diameter, the thickness of the endocardial surface of the left ventricular side of the interventricular septum was included, but the endocardial surface of the left ventricular free wall was not included. At least five cardiac cycles were averaged for each variable, and up to ten cycles were averaged for sinus arrhythmia or atrial fibrillation (Bett and Dryburgh, 1981). No measurements were made from ectopic beats or cardiac cycles immediately following ectopic beats (postextrasystolic potentiated beats).

#### *3.3.4.1 M-mode calculated indices*

In addition, a number of derived indices were calculated. Left ventricular diastolic and systolic diameters were normalised for body surface area (LVDd index and LVDs index respectively), to facilitate comparisons between the three breeds,

which had significantly different body surface areas. Left ventricular fractional shortening (FS%) was calculated according to the following formula:

Formula 3.1

$$FS\% = \frac{(LVDd - LVDs) \times 100}{LVDd}$$

Percentage posterior wall thickening (%PWth) was calculated according to the following formula:

Formula 3.2

$$\%PWth = \frac{(LVPWs - LPWd) \times 100}{LVPWd}$$

Velocity of circumferential fibre shortening (Vcf) can be derived entirely from left ventricular and aortic valve M-mode measurements, and is calculated according to the following formula:

Formula 3.3

$$Vcf = \frac{(LVDd - LVDs)}{LVDd \times \text{ejection time (s)}}$$

Left ventricular ejection time can be obtained from an M-mode recording of the aortic valve, measuring from the start of aortic valve opening until aortic valve closure. An alternative means of measuring left ventricular ejection time is from the spectral Doppler waveform of the aortic blood flow velocity, measuring from the onset of aortic flow until flow ceases. This was the method used for deriving ejection time and Vcf in these studies.

### 3.3.5 Statistical methods

All values are expressed as mean  $\pm$  SD. Coefficients of variation were calculated for the repeated measures in the two normal GSH pointers by dividing the standard deviation by the mean, and multiplying by 100 to express the result as a percentage. A variable was considered to be adequately repeatable when the coefficient of variation was less than 10%. The values were compared between GSH pointers, Dobermanns and cocker spaniels by one way analysis of variance when data were normally distributed, and by Kruskal-Wallis one way analysis of variance when a test for normality failed. When a significant difference was found between groups, multiple pairwise comparisons were made using a Tukey test for normally distributed data, or Dunn's method following a Kruskal-Wallis test. The significance level was set at  $p < 0.05$ . All statistical analyses were carried out using a proprietary statistical software programme (SigmaStat 2.0, Jandel Scientific, San Rafael, CA, USA). Graphs were plotted using SigmaPlot (Jandel Scientific, San Rafael, CA, USA).

## 3.4 RESULTS

### 3.4.1 German short-haired pointers

The M-mode values for the individual GSH pointers are presented in tables 3.1-3.2, with LV chamber dimensions and LV diameter indexed to body surface area in table 3.1, and derived indices and left atrial dimensions in table 3.2. Recordings were considered of adequate quality for all variables, except aortic and left atrial diameter in dog 7. Mean diastolic LV diameter was  $4.16 \pm 0.39$  cm, and end-diastolic diameter indexed to body surface area was  $4.66 \pm 0.34$  cm/m<sup>2</sup>. Mean systolic LV diameter was  $3.00 \pm 0.4$  cm, and mean end-systolic diameter index was  $3.35 \pm 0.34$  cm/m<sup>2</sup>. Dog 4 had a LV diastolic diameter and a diastolic and systolic LV free wall thickness that exceeded the 95% confidence limits, and dog 7 had a systolic interventricular septal thickness that exceeded the 95% confidence limits. LV fractional shortening ranged from 20.5% to 34.9%, with a mean value of  $28.1 \pm 4.6\%$ . Percentage free wall thickening varied from 20.8% to 59%, with a mean value of  $38.6 \pm 10.6\%$ . Mean Vcf was  $1.43 \pm 0.29$  circs/sec, and mean EPSS was  $0.55 \pm 0.16$ cm. The mean ratio of LV diameter to free wall thickness in diastole was  $3.94 \pm 0.51$ , and the mean ratio in systole was  $2.07 \pm 0.46$ .

Table 3.1 M-mode measurements in individual normal GSH pointers.

Dog	LVDd	EDDI	LVDs	ESDI	IVSd	IVSs	LVPWd	LVPWs
	(cm)	(cm/m <sup>2</sup> )	(cm)	(cm/m <sup>2</sup> )	(cm)	(cm)	(cm)	(cm)
1	4.09	4.65	3.04	3.45	0.91	1.11	0.91	1.33
2	4.54	5.04	3.61	4.01	1.13	1.21	0.96	1.16
3	4.54	4.50	3.54	3.50	1.13	1.41	1.06	1.33
4	5.06*	5.01	3.68	3.64	1.43	1.67	1.50*	2.14*
5	4.19	4.76	2.98	3.39	1.01	1.35	0.97	1.36
6	3.98	5.10	2.59	3.32	0.99	1.31	0.95	1.51
7	4.03	4.86	2.77	3.34	1.38	1.94*	1.04	1.47
8	3.57	4.20	2.43	2.86	1.44	1.68	1.23	1.82
9	4.13	4.39	2.78	2.96	1.13	1.62	1.08	1.39
10	4.34	4.13	3.05	2.90	1.13	1.37	1.18	1.73
11	3.89	4.80	3.04	3.75	1.02	1.26	0.95	1.32
12	4.01	4.95	2.72	3.36	0.90	1.22	0.98	1.32
13	3.72	4.23	2.73	3.10	1.19	1.34	1.12	1.44
mean	4.16	4.66	3.00	3.35	1.14	1.42	1.07	1.49
SD	0.39	0.34	0.40	0.34	0.18	0.24	0.16	0.26

\*Indicates value >2SD from the mean. LV: left ventricle, LVDd: LV diameter in diastole, EDDI: LV end-diastolic diameter index, LVDs: LV diameter in systole, ESDI: LV end-systolic diameter index, IVSd: septal thickness in diastole, IVSs: septal thickness in systole, LVPWd: LV free wall thickness in diastole, LVPWs: LV free wall thickness in systole.

Table 3.2 M-mode measurements (continued) in normal GSH pointers.

Dog	FS	PWth	Vcf	EPSS	Ao	LA	LA:Ao	Rad/thD	Rad/thS
	(%)	(%)	(circ/s)	(cm)	(cm)	(cm)	(cm)		
1	25.7	46.2	1.24	0.57	2.25	2.42	1.08	4.49	2.29
2	20.5	20.8	0.98	0.73	2.23	2.14	0.96	4.73	3.11
3	22.0	25.5	1.06	0.64	2.30	2.18	0.95	4.28	2.66
4	27.3	42.7	1.43	0.55	2.31	2.49	1.08	3.37	1.72
5	28.9	40.2	1.54	0.72	2.21	2.08	0.94	4.32	2.19
6	34.9	59.0	1.95	0.34	1.98	2.19	1.11	4.19	1.72
7	31.3	41.4	1.63	0.47	NR	NR	NR	3.88	1.88
8	31.9	48.0	1.81	0.63	2.16	2.48	1.15	2.90	1.34
9	32.7	28.7	1.57	0.63	2.27	2.34	1.03	3.82	2.00
10	29.7	46.6	1.44	0.69	2.46	2.45	1.00	3.68	1.76
11	21.9	39.0	1.11	0.60	2.09	2.31	1.11	4.09	2.30
12	32.0	34.7	1.62	0.25	2.27	2.37	1.04	4.09	2.06
13	26.6	28.6	1.25	0.29	2.00	2.33	1.17	3.32	1.90
mean	28.1	38.6	1.43	0.55	2.21	2.32	1.05	3.94	2.07
SD	4.6	10.6	0.29	0.16	0.14	0.14	0.08	0.51	0.46

\*Indicates value >2SD from the mean. FS%: left ventricular fractional shortening, PWth: percentage left ventricular free wall thickening, EPSS: mitral valve E point to septal separation, Vcf: velocity of circumferential fibre shortening, LA: left atrial diameter, Ao: aortic diameter, circ/s: circumferences /second, Rad/thD: ratio of LV diameter to free wall thickness in diastole, Rad/thS: ratio of LV diameter to free wall thickness in systole.

### 3.4.1.1 Reproducibility

Repeated measurements in two GSH pointers shown in tables 3.3-3.5. Coefficients of variation for the repeated measures varied from 1.6% for left ventricular diameter in diastole in dog 2, to 28% for percentage posterior wall thickening in dog 1. Coefficients of variation were less than 10% for both dogs in

the following variables: LVDd, LVDs, IVSs; LVPWd, LVPWs, FS, Vcf, aortic diameter, LA diameter and LA:Ao.

Table 3.3. Repeated M-mode values for normal GSH pointers.

	LVDd (cm)		LVDs (cm)		IVSd (cm)		IVSs (cm)		LVPWd (cm)		LVPWs (cm)	
	Dog 1	Dog 2	Dog 1	Dog 2	Dog 1	Dog 2	Dog 1	Dog 2	Dog 1	Dog 2	Dog 1	Dog 2
Day 1	4.21	3.72	2.94	2.73	0.94	1.19	1.2	1.34	0.87	1.12	1.02	1.44
Day 2	4.01	3.71	2.72	2.83	0.9	0.85	1.22	1.09	0.98	1.00	1.32	1.38
Day 3	4.2	3.66	3.13	2.88	0.88	0.80	1.24	1.06	0.86	0.96	1.09	1.29
Day 4	3.93	3.59	2.8	2.75	0.88	0.86	1.05	1.11	0.95	1.02	1.29	1.34
Day 5	4.01	3.6	2.93	2.73	0.87	0.90	1.31	1.10	0.93	1.01	1.18	1.36
mean	4.07	3.66	2.90	2.78	0.89	0.92	1.20	1.14	0.92	1.02	1.18	1.36
SD	0.13	0.06	0.14	0.07	0.05	0.16	0.11	0.11	0.05	0.06	0.12	0.05
CV%	3.1	1.6	4.9	2.4	5.6	16.9	9.5	9.9	5.3	5.8	9.8	4.0

LV: left ventricle, LVDd: LV diameter in diastole, LVDs: LV diameter in systole, IVSd: septal thickness in diastole, IVSs: septal thickness in systole, LVPWd: LV free wall thickness in diastole, LVPWs: LV free wall thickness in systole, CV%: coefficient of variation.

Table 3.4 Repeated M-mode values for normal GSH pointers (continued).

	EPSS (cm)		Ao (cm)		LA (cm)	
	Dog 1	Dog 2	Dog 1	Dog 2	Dog 1	Dog 2
Day 1	0.29	0.29	2.19	2.00	2.11	2.33
Day 2	0.25	0.33	2.27	2.00	2.37	2.25
Day 3	0.30	0.22	2.05	2.07	1.97	2.11
Day 4	0.28	0.26	2.20	2.10	2.12	2.20
Day 5	0.29	0.32	2.20	1.96	2.22	2.01
mean	0.28	0.28	2.18	2.03	2.16	2.18
SD	0.02	0.05	0.07	0.06	0.19	0.12
CV%	6.6	15.9	3.4	2.8	9.0	5.7

LEPSS: mitral valve E point to septal separation, Vcf: velocity of circumferential fibre shortening, LA: left atrial diameter, Ao: aortic diameter, CV%: coefficient of variation.

Table 3.5 Repeated derived M-mode values for normal GSH pointers.

	FS (%)		PWth (%)		Vcf (circ/s)		LA:Ao (cm)	
	Dog 1	Dog 2	Dog 1	Dog 2	Dog 1	Dog 2	Dog 1	Dog 2
Day 1	29.9	26.4	17.2	28.57	1.53	1.25	0.96	1.17
Day 2	32.1	23.2	34.7	38.00	1.62	1.13	1.04	1.13
Day 3	25.4	21.0	26.7	34.38	1.26	0.98	0.96	1.02
Day 4	28.8	23.2	35.8	31.37	1.43	1.09	0.96	1.05
Day 5	26.7	24.0	26.9	34.65	1.32	1.13	1.01	1.02
mean	28.6	23.6	28.3	33.39	1.43	1.12	0.99	1.08
SD	2.6	1.9	7.9	3.57	0.14	0.10	0.07	0.07
CV%	9.2	8.2	28.0	10.7	9.8	8.8	6.9	6.1

FS%: left ventricular fractional shortening, PWth: percentage left ventricular free wall thickening, EPSS: mitral valve E point to septal separation, Vcf: velocity of circumferential fibre shortening, LA: left atrial diameter, Ao: aortic diameter, circ/s: circumferences / second, CV%: coefficient of variation.

### 3.4.2 Dobermanns and cocker spaniels with DCM

The values for Dobermanns are displayed in tables 3.6-3.7, and for cocker spaniels in tables 3.8-3.9. Adequate recordings were not obtained for mitral valve E-point to septal separation in Dobermanns 4 and 10; or for aortic and left atrial diameters in Dobermanns 2, 3, 4, 5, 7, 9 and 19, and cocker spaniels 1, 2, 4, 5 and 10. This was generally a result of too few cardiac cycles being recorded, rather than technical difficulties in obtaining a satisfactory image.

In the affected Dobermanns, mean LV diastolic diameter was 6.53 cm (range: 5.63 cm to 7.64 cm), and mean LV systolic diameter was 5.92 cm (range: 5.0 cm to 6.56 cm). Mean end-diastolic diameter index was  $5.92 \pm 0.55$  cm, and mean end-systolic diameter index was  $5.37 \pm 0.66$  cm. Both septal and free walls were thin, averaging 0.77 cm and 0.76 cm respectively, in diastole. Free wall thickness in



systole exceeded the 95% confidence limits in dog 9. Mean LV fractional shortening was 9.12% (range: 4.7 to 15.4%), with dog 5 having the highest value (which exceeded the 95% confidence limits). A typical M-mode recording at the mitral valve level is shown in figure 3.1, demonstrating increased EPSS.

Table 3.6: M-mode values from Dobermanns with DCM

Dog	LVDd	EDDI	LVDs	ESDI	IVSd	IVSs	LVPWd	LVPWs
	cm	cm/m <sup>2</sup>	cm	cm/m <sup>2</sup>	cm	cm	cm	cm
1	6.98	6.84	6.47	6.34	0.94	1.10	0.67	0.86
2	5.74	5.98	5.03	5.23	0.69	0.80	0.76	0.83
3	5.63	5.86	5.00	5.21	0.81	1.05	0.87	0.86
4	6.89	4.92	6.25	4.46	0.77	0.94	1.01	0.95
5	7.64	5.97	6.46	5.05	0.70	1.09	0.63	0.7
6	6.79	6.06	6.22	5.55	0.63	0.77	0.62	0.69
7	6.12	5.94	5.76	5.59	0.56	0.69	0.67	0.73
8	6.47	6.10	5.95	5.61	0.68	0.81	0.76	0.85
9	7.2	7.3	6.4	6.6	0.9	1.2	0.9	1.1*
10	6.7	5.3	6.2	4.8	1.0	1.1	0.8	0.8
11	5.68	4.85	5.41	4.62	0.76	0.78	0.64	0.66
mean	6.53	5.92	5.92	5.37	0.77	0.95	0.76	0.83
SD	0.67	0.74	0.55	0.66	0.13	0.18	0.13	0.13

\*indicates value >2 SD from the mean. LV: left ventricle, LVDd: LV diameter in diastole, EDDI: end-diastolic diameter index, LVDs: LV diameter in systole, ESDI: end-systolic diameter index, IVSd: septal thickness in diastole, IVSs: septal thickness in systole, LVPWd: LV free wall thickness in diastole, LVPWs: LV free wall thickness in systole.

Table 3.7: Mode values from Dobermanns with DCM (continued)

Dog	FS %	PWth %	Vcf circ/s	EPSS cm	Ao cm	LA cm	LA:Ao cm	Rad/thD	Rad/thS
1	7.3	22.1	0.51	2.57	2.13	2.96	1.39	10.42	7.52
2	12.0	8.4	0.87	1.12	NR	NR	NR	7.55	6.06
3	11.2	-1.1	0.71	1.79	NR	NR	NR	6.47	5.81
4	9.1	-5.9	0.57	NR	NR	NR	NR	6.82	6.58
5	15.4*	10.0	1.03	2.18	NR	NR	NR	12.13	9.23
6	8.3	10.1	0.55	1.42	2.53	3.47	1.37	10.95	9.01
7	5.6	8.2	0.33	0.82	NR	NR	NR	9.13	7.89
8	7.9	10.6	0.42	2.5	2.54	3.71	1.46	8.51	7.00
9	10.5	16.0	0.97	2.15	NR	NR	NR	7.65	5.74
10	8.4	8.3	0.6	NR	NR	NR	NR	8.74	7.33
11	4.70	3.0	0.34	1.27	2.55	4.54	1.78	8.88	8.20
mean	9.12	8.15	0.63	1.76	2.44	3.67	1.50	8.84	7.31
SD	3.03	7.61	0.24	0.63	0.21	0.66	0.19	1.75	1.21

\*indicates value >2 SD from the mean. FS%: left ventricular fractional shortening, PWth: percentage left ventricular free wall thickening, Vcf: velocity of circumferential fibre shortening, circ/s: circumferences / second, EPSS: mitral valve E point to septal separation, Ao: aortic diameter, LA: left atrial diameter, LA:Ao: ratio of left atrium to aortic diameter, Rad/thD: ratio of LV diameter to free wall thickness in diastole, Rad/thS: ratio of LV diameter to free wall thickness in systole.

Table 3.8: M-mode values from Cocker spaniels with DCM

Dog	LVDd cm	EDDI cm/m <sup>2</sup>	LVDs cm	ESDI cm/m <sup>2</sup>	IVSd cm	IVSs cm	LVPWd cm	LVPWs cm
1	5.49	9.47	5.1	8.64	0.55	0.68	0.59	0.70
2	6.29	12.09	5.29	10.17	0.62	0.7	0.61	0.86
3	5.8	9.21	5.22	8.28	0.47	0.63	0.54	0.61
4	5.37	8.95	4.76	7.93	0.67	0.67	0.90	1.04
5	3.81*	5.95	3.44*	5.37	0.87	1.01	0.81	1.07
6	5.18	7.29	4.97	7.00	0.54	0.64	0.67	0.68
7	5.34	8.90	4.84	8.06	0.51	0.53	0.55	0.65
8	5.2	9.28	4.69	8.37	0.56	0.62	0.69	0.97
9	5.29	9.12	4.89	8.43	0.63	0.77	0.58	0.61
10	6.21	10.35	5.69	9.48	0.55	0.65	0.62	0.75
11	4.82	7.77	4.40	7.10	1.03	1.14	0.80	1.29
mean	5.35	8.94	4.84	8.08	0.64	0.73	0.67	0.82
SD	0.67	1.59	0.58	1.28	0.17	0.18	0.12	0.19

\*indicates value >2 SD from the mean. LV: left ventricle, LVDd: LV diameter in diastole, LVDs: LV diameter in systole, IVSd: septal thickness in diastole, IVSs: septal thickness in systole, LVPWd: LV free wall thickness in diastole, LVPWs: LV free wall thickness in systole.

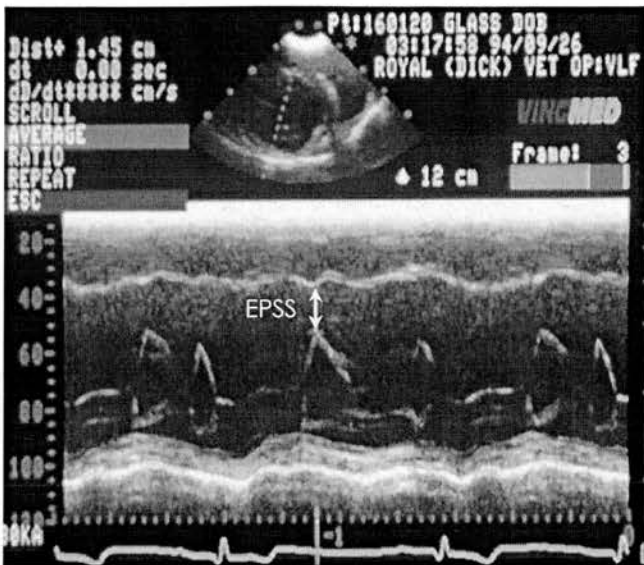
Table 3.9: M-mode values from Cocker spaniels with DCM (continued)

Dog	FS	PWth	Vcf	EPSS	Ao	LA	LA:Ao	Rad/thD	Rad/thS
	%	%	circ/s	cm	cm	cm	cm		
1	6.9	15.7	0.45	2.2	NR	NR	NR	9.31	7.29
2	16.0*	29	1.24*	2.35	NR	NR	NR	10.31	6.15
3	10.1	11.4	0.81	2.32	1.85	2.93	1.59	10.74	8.56
4	11.2	13.4	0.77	2.15	NR	NR	NR	5.97	4.58
5	8.4	24.2	0.61	0.93	NR	NR	NR	4.70	3.21
6	4.0	1.4	0.23	1.7	1.68	2.19	1.3	7.73	7.31
7	9.4	15.4	0.64	1.94	1.84	2.37	1.29	9.71	7.45
8	9.8	28.9	0.65	1.95	1.92	2.18	1.13	7.54	4.84
9	7.4	5.2	0.57	1.95	1.4	2.31	1.65	9.12	8.02
10	7.2	21.0	0.41	3.32*	NR	NR	NR	10.02	7.59
11	8.6	31.2	0.58	1.71	2.08	2.72	1.31	6.03	4.19
mean	9.0	17.9	0.63	2.05	1.80	2.45	1.38	8.29	6.50
SD	3.03	9.90	0.26	0.58	0.23	0.31	0.20	2.03	1.74

\*indicates value >2 SD from the mean. FS%: left ventricular fractional shortening, PWth: percentage left ventricular free wall thickening, Vcf: velocity of circumferential fibre shortening, circ/s: circumferences / second, EPSS: mitral valve E point to septal separation, Ao: aortic diameter, LA: left atrial diameter, LA:Ao: ratio of left atrium to aortic diameter, Rad/thD: ratio of LV diameter to free wall thickness in diastole, Rad/thS: ratio of LV diameter to free wall thickness in systole.

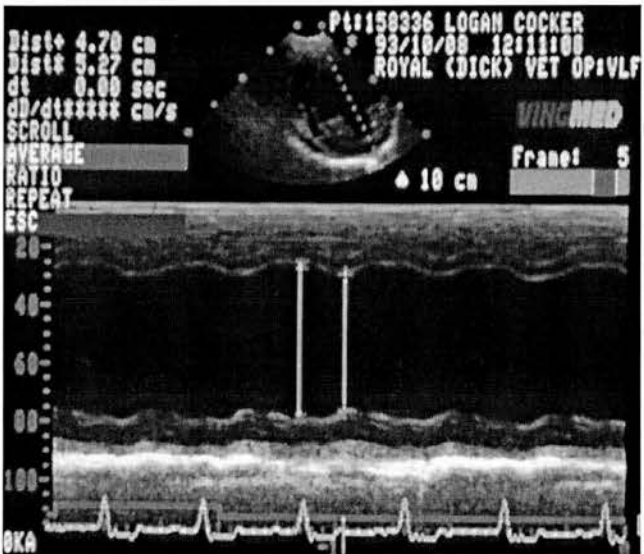
In the affected cocker spaniels, mean LV diastolic diameter was 5.35 cm (range: 3.81 cm to 6.29 cm), and mean LV systolic diameter was 4.84 cm (range: 3.44 cm to 5.69 cm). Dog 5 had a LV diameter less than the 95% confidence limits in both diastole and systole. Mean end-diastolic diameter index was  $8.94 \pm 1.59$  cm, and mean end-systolic diameter index was  $8.08 \pm 1.28$  cm. Mean LV fractional shortening was 9.0% (range: 4.0 to 16.0%), and mean velocity of circumferential shortening was 0.63 circs/sec (range: 0.23 to 1.24). Dog 2 had the highest value for both these indices, and exceeded the 95% confidence limits with both. A typical M-mode recording of a cocker spaniel at the chordal level is illustrated in figure 3.2.

Figure 3.1: M-mode at mitral valve level.



M-mode echocardiogram recorded at the mitral valve level in Dobermann 8, showing increased E-point to septal separation (EPSS).

Figure 3.2: M-mode of left ventricle.



M-mode echocardiogram recorded at the level of the chordae tendineae in cocker 4, showing increased left ventricular dimensions, thin walls relative to chamber diameter, and poor wall motion.

### 3.4.3 Comparison of GSH pointers and dogs with DCM

A summary of the mean values for each group (normal GSH pointers, Dobermanns with DCM, and cocker spaniels with DCM) is displayed in table 3.10, with the results of comparisons between the groups. All variables showed highly significant differences between the groups ( $p < 0.001$ ), with pairwise comparisons showing the dogs with DCM to be significantly different from the normal dogs for all the variables except aortic diameter (Dobermanns not different from GSH pointers) and LA diameter ( $p < 0.008$  between groups; cocker spaniels not different from GSH pointers).

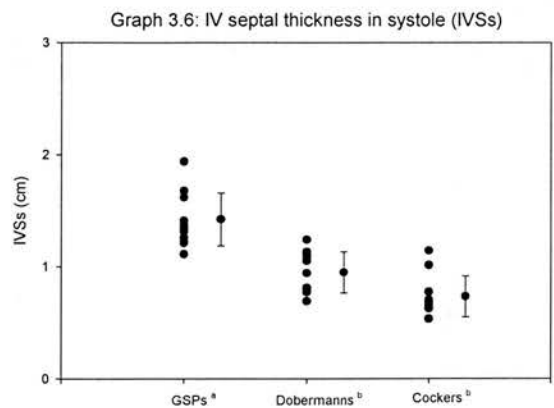
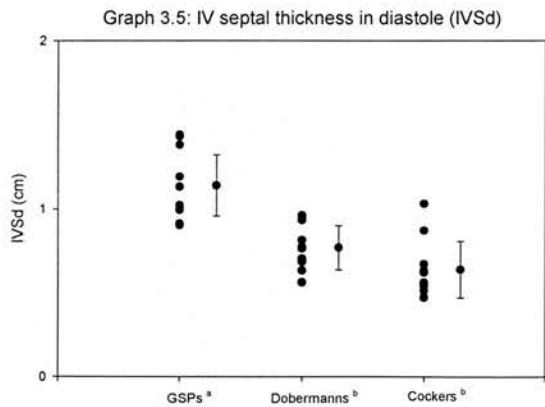
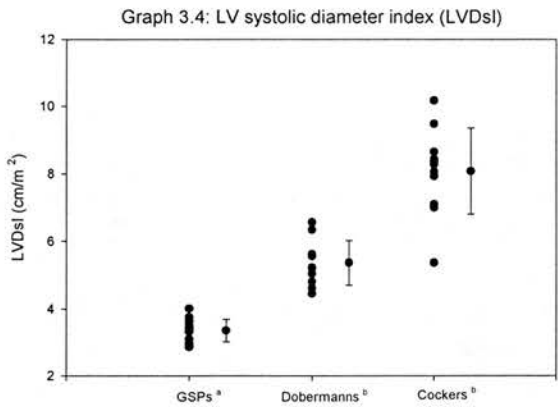
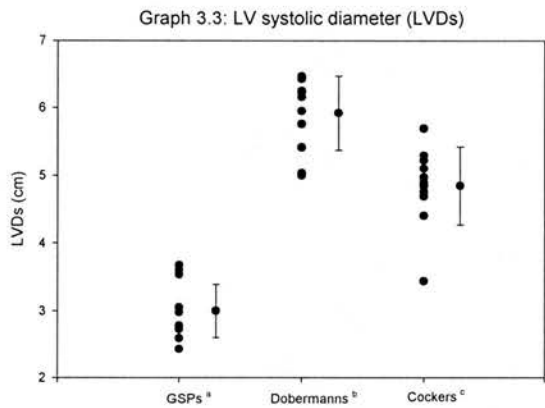
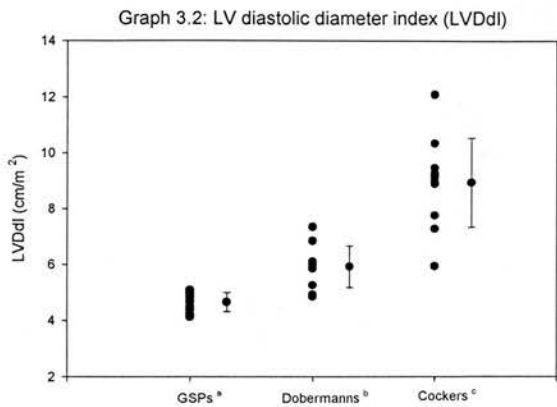
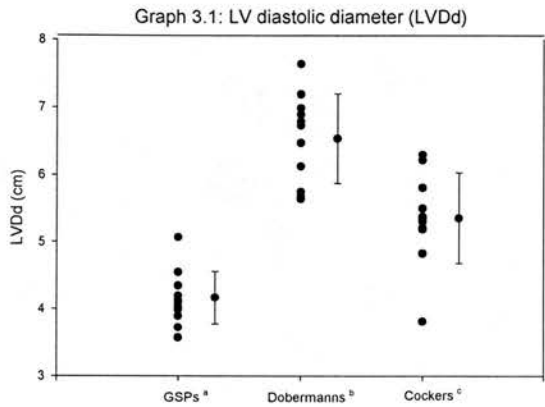
The raw data for the three groups for all variables are displayed in graphs 3.1-3.17. Dobermanns with DCM showed differences from cocker spaniels with DCM for the following variables: LVDd, LVDd index and LVDs and Ao. Although Dobermanns had a significantly higher mean LVDd (graph 3.1), the cocker spaniels had a significantly higher mean LVDd index (graph 3.2). Dobermanns had a significantly higher LVDs than cocker spaniels (graph 3.3), but there was no significant difference between the LVDs index between the two groups (graph 3.4). Aortic diameter was smaller in the cocker spaniels than the Dobermanns (graph 3.13), but there were no significant differences between the Dobermanns and the cocker spaniels for any of the other variables.

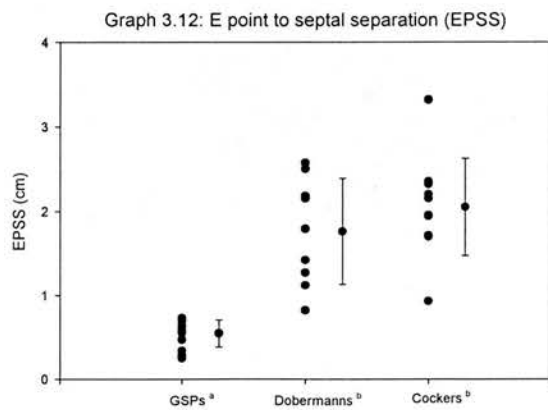
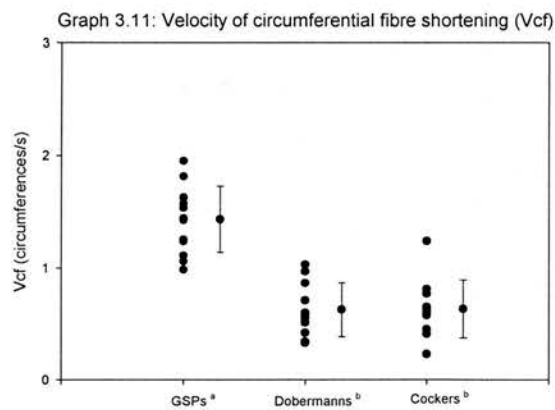
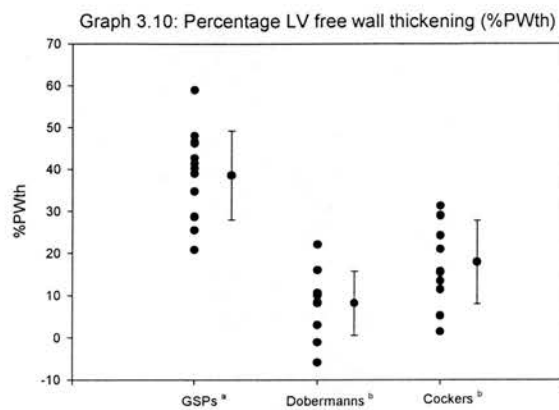
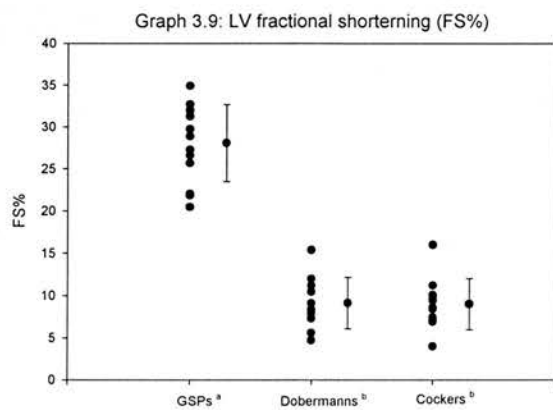
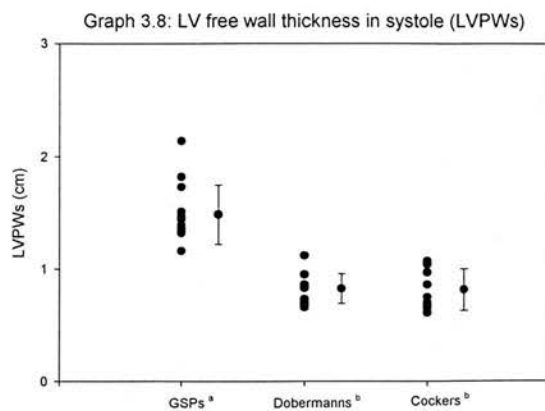
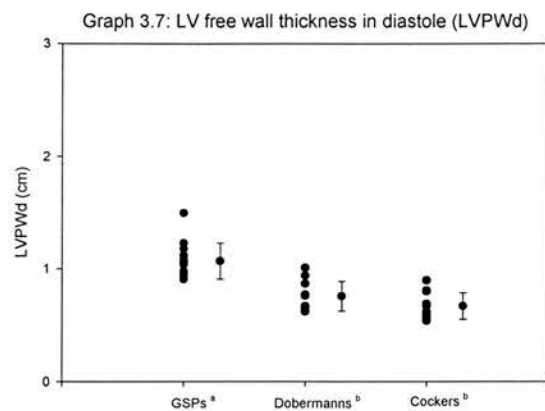
Table 3.10: Group mean M-mode values for GSH pointers, Dobermanns and Cocker spaniels, with results of group comparisons.

Variable	units	GSPs		Dobes		Cockers		ANOVA P value	Significant differences		
		mean	SD	mean	SD	mean	SD		Dob vs GSP	CSp vs GSP	Dob vs CSp
LVDd	cm	4.16	0.39	6.53	0.66	5.35	0.67	<0.001	+	+	+
LVDdI	cm/m <sup>2</sup>	4.66	0.34	5.96	0.52	8.94	1.59	<0.001	+	+	+
LVDs	cm	3.00	0.40	5.89	0.59	4.84	0.58	<0.001	+	+	+
LVDsI	cm/m <sup>2</sup>	3.35	0.34	5.38	0.54	8.08	1.28	<0.001	+	+	-
IVSd	cm	1.14	0.18	0.72	0.12	0.64	0.17	<0.001	+	+	-
IVSs	cm	1.42	0.24	0.91	0.16	0.73	0.18	<0.001	+	+	-
LVPWd	cm	1.07	0.16	0.75	0.13	0.67	0.12	<0.001	+	+	-
LVPWs	cm	1.49	0.26	0.81	0.09	0.82	0.19	<0.001	+	+	-
FS	%	25.67	8.82	9.60	3.12	9.00	3.03	<0.001	+	+	-
PWth	%	38.55	10.61	7.80	8.37	17.9	9.9	<0.001	+	+	-
Vcf	circs/s	1.43	0.29	0.62	0.23	0.63	0.26	<0.001	+	+	-
EPSS	cm	0.55	0.16	1.77	0.68	2.05	0.58	<0.001	+	+	-
LA	cm	2.32	0.14	3.67	0.66	2.45	0.31	<0.008	+	-	-
Ao	cm	2.21	0.14	2.44	0.21	1.80	0.23	<0.001	-	+	+
LA:Ao		1.05	0.08	1.5	0.19	1.38	0.20	<0.001	+	+	-
Rad/thD		3.94	0.51	8.84	1.75	8.29	2.03	<0.001	+	+	-
Th/RadD		0.27	0.04	0.12	0.02	0.12	0.04	<0.001	+	+	-
Rad/thS		2.07	0.46	7.31	1.21	6.5	1.74	<0.001	+	+	-

GSPs: German short-haired pointers, Dobes: Dobermanns, Dob: Dobermann, CSp: cocker spaniel, LV: left ventricle, LVDd: LV diameter in diastole, LVDdI: LV diastolic diameter index, LVDs: LV diameter in systole, LVDsI: LV systolic diameter index, IVSd: septal thickness in diastole, IVSs: septal thickness in systole, LVPWd: LV free wall thickness in diastole, LVPWs: LV free wall thickness in systole, FS%: left ventricular fractional shortening, PWth: percentage left ventricular free wall thickening, EPSS: mitral valve E point to septal separation, Vcf: velocity of circumferential fibre shortening, LA: left atrial diameter, Ao: aortic diameter, circ/s: circumferences / second, Rad/thD: LV diastolic diameter/LV diastolic free wall thickness, Th/RadD: LV diastolic free wall thickness /LV diastolic diameter, Rad/thS: LV systolic diameter/LV systolic free wall thickness.

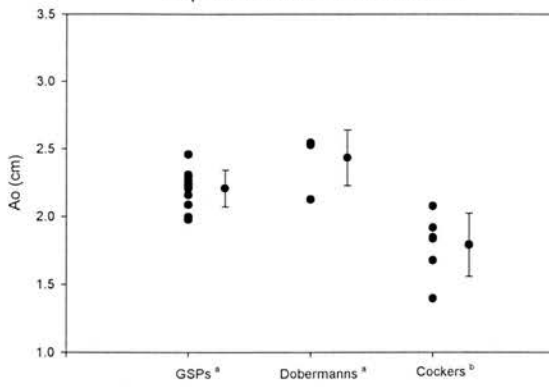
Comparison of M-mode values in normal dogs and dogs with DCM



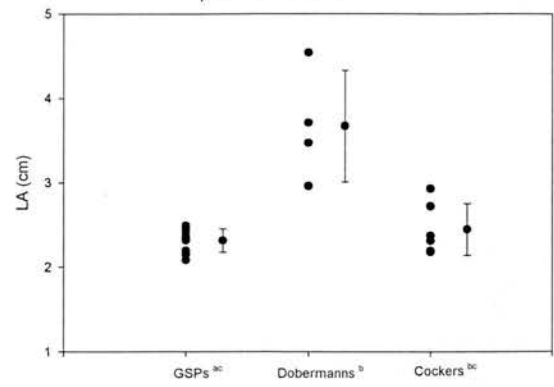




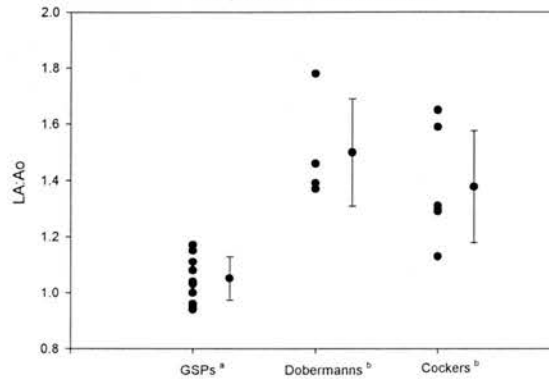
Graph 3.13: M-mode aortic diameter



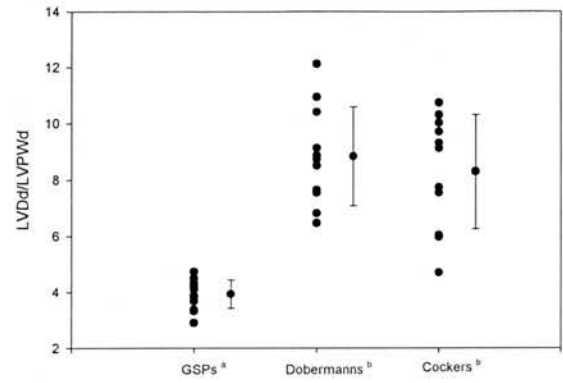
Graph 3.14: M-mode LA diameter



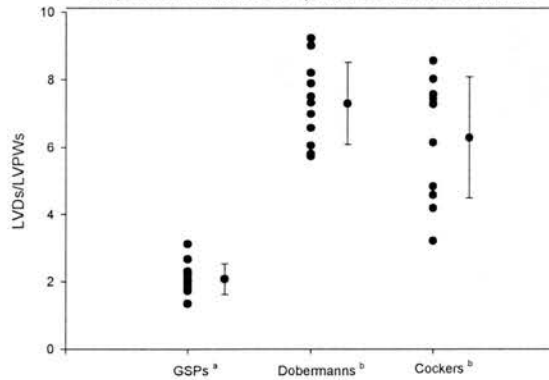
Graph 3.15: M-mode LA:Ao



Graph 3.16: M-mode LV diastolic radius/free wall ratio



Graph 3.17: M-mode LV systolic radius/ free wall ratio



When German short-haired pointers (GSPs), Dobermanns or English cocker spaniels (cockers) were statistically different following analysis of variance, a different letter in superscript is ascribed (eg. GSP<sup>a</sup> and Dobermann<sup>b</sup> indicates the two groups were significantly different at the  $p < 0.05$  level in pairwise comparisons)

LV: left ventricle, LVDd: LV diameter in diastole, LVDdI: LV diastolic diameter index, LVDs: LV diameter in systole, LVDsI: LV systolic diameter index, IVSd: septal thickness in diastole, IVSs: septal thickness in systole, LVPWd: LV free wall thickness in diastole, LVPWs: LV free wall thickness in diastole, FS%: left ventricular fractional shortening, PWth: percentage left ventricular free wall thickening, EPSS: mitral valve E point to septal separation, Vcf: velocity of circumferential fibre shortening, LA: left atrial diameter, Ao: aortic diameter, circ/s: circumferences / second.

### 3.5 DISCUSSION

#### 3.5.1 Normal GSH pointers

Although the reproducibility of M-mode variables has received close attention in human studies, there have been few reports of the reproducibility of 2D-guided M-mode measurements in conscious dogs. The coefficients of variation (CV%) obtained in this study were relatively small for most variables, and were less than 5% in the measurements of left ventricular diameters. Only three variables had coefficients of variation which exceeded 10%: IVSd in dog 2 (16.9%), %PWth in both dogs (28% and 10.7%), and EPSS in dog 2 (15.9%).

Although 2D echocardiography is often used for the subjective diagnosis of canine DCM, M-mode echocardiography is still currently considered the non-invasive technique of choice for the objective assessment of ventricular function in affected dogs. Most echocardiographic studies of canine DCM have concentrated on M-mode variables, and most clinicians place considerable reliance on the left ventricular fractional shortening for assessing systolic function in DCM. In this study, dogs affected with DCM were readily separated from the normal dogs on the basis of M-mode values, with comparatively little overlap in most of the variables, particularly when body surface area indices were calculated.

**Table 3.11: Comparison of previously published normal M-mode derived indices with GSH pointer values**

value	reference	n	BW kg	FS %	PWth%	Vcf Circs/sec	EPSS mm
GSP	Present study	13	26.9 ± 3.8	28.10 (18.9-37.3)	38.6 (17.3-59.8)	1.43 (0.85-2.02)	5.47 (2.2-8.7)
mixed	(Boon <i>et al.</i> , 1983)	20	19.3	36.26 (33.6-38.9)	61.7 (55.2-68.3)	2.07 (1.58-2.79)	
mixed	(Lombard, 1984b)	40	24.0 ± 10.0	39.00 (27-51)			
mixed	(DeMadron, 1983)	27	11.1 ± 8.7	30.66 (15.5-45.9)	56.00 (17.2-94.8)	1.96 (1.16-2.76)	6.44 (0.1-12.8)
mixed	(Smucker <i>et al.</i> , 1990)	31	24 ± 10		36.00 (16-56)		
mixed	(Mashiro <i>et al.</i> , 1976)	16	17.6 ± 3.1	30.83			
greyhound	(Snyder <i>et al.</i> , 1995b)	11	29.1 ± 3.7	28.80 (20.4-37.2)		1.60 (1.02-2.18)	
greyhound	(Page <i>et al.</i> , 1993)	16	26.6 ± 3.5	25.30 (12.7-37.9)	26.45		
Afghan*	(Morrison <i>et al.</i> , 1992)	20	23 (17-36)	33.00 (24-48)			
G Ret*	(Morrison <i>et al.</i> , 1992)	20	32 (23-41)	39.00 (27-55)			
Spanish Mastiff	(Bayon <i>et al.</i> , 1994)	12	52.4 ± 3.3	39.20	56.70		6.70 (6.1-7.3)
Newf*	(Koch <i>et al.</i> , 1996)	27	61 (47-69.5)	30.00 (22-37)	31.00 (11-40)	1.70 (1.1-2.5)	6.00 (3-14)
IWH*	(Koch <i>et al.</i> , 1996)	20	68.5 (50-80)	28.00 (20-34)	24.00 (10-38)	1.70 (1.0-2.2)	7.00 (1-10)
Great Dane*	(Koch <i>et al.</i> , 1996)	15	62 (52-75)	25.00 (18-36)	18.00 (-9 – 29)	1.70 (1.0-2.3)	8.00 (5-12)

Results are expressed as means ± SD; or (95% confidence intervals); or (range) where breed is suffixed with an asterisk. GSP: German short-haired pointer, G Ret: golden retriever, Newf: Newfoundland, IWH: Irish wolfhound, FS%: left ventricular fractional shortening, %PWth: percentage left ventricular free wall thickening, EPSS: mitral valve E point to septal separation, Vcf: velocity of circumferential fibre shortening.

### 3.5.2 Comparison of results in normal dogs with other studies

Some of the variables in the normal group of pointers differed from published normal values (see table 3.11). A left ventricular fractional shortening of less than 25% has been used as an inclusion criterion for studies of dilated cardiomyopathy (Monnet *et al.*, 1995) (Tidholm and Jonsson, 1996), yet three of the pointers had FS% values below this level (GSPs 2, 3 and 11). Low values for FS% have been reported in greyhounds. Page and co-workers (Page *et al.*, 1993) published a mean value of  $25.4 \pm 6.3\%$  for FS% in normal unsedated greyhounds, and a study of normal greyhounds by Snyder and others (Snyder *et al.*, 1995b) also reported values for FS% which fell outside the 95% confidence limits established by Boon and others (Boon *et al.*, 1983; Boon, 1998b).

One possible explanation is that many of the early reports of normal canine M-mode values were based on unguided M-mode studies. Hoenecke and others (1982) showed that estimates of FS% were artifactually increased using unguided M-mode when the cursor deviated from the meridian (Hoenecke *et al.*, 1982). When M-mode studies are recorded using 2D-guided steering, this type of deviation is less likely. It is possible that values for FS% obtained using 2D-guided M-mode are more accurate, though lower.

Several of the GSH pointers were physically trained to a greater degree than commonly found in domestic pets, although exercise-training is not likely to explain the low FS% seen in these GSH pointers or in greyhounds in general. Reduced ejection fraction and Vcf have been reported in endurance athletes (Pavlik *et al.*, 1986), but other studies of endurance athletes has either shown a non-significant trend towards reduced systolic function (Gilbert *et al.*, 1977) or no obvious

differences with normal subjects (Douglas *et al.*, 1986). The commonest finding with physical training in humans is an increase in LV wall thickness (Pelliccia *et al.*, 1991; Urhausen *et al.*, 1996), with or without a slight increase in LV diastolic diameter (Fagard *et al.*, 1984; Missault *et al.*, 1993). Invasive and necropsy findings in exercise-trained dogs appear to be similar, with an increase in LV wall thickness and no change in systolic function (Carew and Covell, 1978; Mackintosh *et al.*, 1983; Wyatt and Mitchell, 1974). The effects of training in greyhounds have been examined by M-mode echocardiography, and again the result was an increase in LV wall thickness, with no change in FS% (Lonsdale *et al.*, 1998).

The values for Vcf in the normal dogs were also low ( $1.43 \pm 0.29$  circs/s) compared with those published by Atkins ( $2.48 \pm 0.5$ ), Calvert ( $2.07 \pm 0.16$ ) and Boon ( $2.1 \pm 0.37$ ) (Atkins and Snyder, 1992b) (Calvert and Brown, 1986) (Boon *et al.*, 1983). Once again, the results in normal greyhounds reported by Snyder were also low ( $1.6 \pm 0.29$ ) (Snyder *et al.*, 1995), although this is to be expected as Vcf is derived from FS%.

It has previously been recognised that values of %PWth in dogs was often lower than in humans (Feneley and Hickie, 1984). Published normal values in conscious dogs show a wide range, from  $31.9 \pm 2.9$  % (Sasayama *et al.*, 1976) to  $61.7 \pm 14$  % (Boon *et al.*, 1983). In this study, the normal pointers had values which are consistent with the lower end of published normal ranges ( $38.6 \pm 10.6$  %), and they were higher than the values published for normal greyhounds by Page (26%) (Page *et al.*, 1993).

The lower than previously reported values for FS% and Vcf in the normal dogs in this study should at least prompt caution in rendering a diagnosis of DCM in

asymptomatic dogs based on reduced FS% alone. At the very least, one should exercise caution in extrapolating M-mode values that are associated with development of DCM in Dobermanns, to other breeds. None of the GSH pointers in this study have developed DCM at the time of writing (at up to six years follow-up), nor is it likely that many of the greyhounds in Snyder's and Page's studies have done so (Page *et al.*, 1993; Snyder *et al.*, 1995). At present, it would seem that it might indeed be necessary to compile normal canine M-mode reference values on a breed-specific basis.

### 3.5.3 Cocker spaniels with DCM

The values in the cocker spaniels with DCM differ somewhat from those published previously by Gooding and others in 1986. A comparison of the values is listed in table 3.12 (Gooding *et al.*, 1986b). Mean LVDd in this study was  $5.3 \pm 0.7$  cm (versus  $4.0 \pm 0.5$  cm in Gooding's study), mean LVDs was  $4.8 \pm 0.7$  cm (versus  $3.0 \pm 0.6$  cm), IVSd was  $0.59 \pm 0.11$  cm (versus  $0.82 \pm 0.16$  cm) and LVPWd was  $0.65 \pm 0.11$  cm (versus  $0.86 \pm 0.13$  cm). Thus the cocker spaniels in this study had a greater degree of ventricular dilation, and thinner ventricular walls. The inclusion criteria for selection of dogs affected with DCM in Gooding's study were based on ECG or radiographic evidence of cardiac enlargement, rather than development of overt cardiac failure as in the present study. It seems likely that the dogs in the present study had more advanced disease than in Gooding's study.

One of the cockers (dog 5) had a localised area of hypertrophy affecting the interventricular septum, as described for one dog in the clinico-pathological study in

Gooding's report (Gooding *et al.*, 1982). This dog also had the smallest LVDd, but a FS% of 8.4%.

Table 3.12: Comparison of DCM M-mode values with previously published values

	Cocker spaniels				Dobermanns			
	Present study		Gooding <i>et al.</i> , 1986		Present study		Calvert & Brown, 1986	
	<i>mean</i>	$\pm SD$	<i>mean</i>	$\pm SD$	<i>mean</i>	$\pm SD$	<i>mean</i>	$\pm SD$
LVDd (cm)	5.34	0.68	4.0	0.52	6.53	0.67	5.76	1.09
LVDs (cm)	4.78	0.67	3.0	0.62	5.92	0.55	4.69	1.17
FS%	10.3	5.1	25.4	5.75	9.12	3.03	18.3	5.91
IVSd (cm)	0.59	0.11	0.82	0.16	0.77	0.13	0.79	0.12
IVSs (cm)	0.68	0.12			0.95	0.18	0.99	0.14
LVPWd (cm)	0.65	0.11	0.86	0.13	0.76	0.13	0.81	0.09
LVPWs (cm)	0.8	0.17			0.83	0.13	1.01	0.11
PWth%	19	12.1			8.15	7.61	19.95	5.85
Vcf (circ/s)	0.7	0.34			0.63	0.24	1.36	0.31
EPSS (cm)	1.97	0.67			1.76	0.63	1.72	0.86
LA (cm)	2.48	0.34			3.67	0.66	4.07	0.77
Ao (cm)	1.72	0.19			2.44	0.21	2.66	0.87
LA:Ao	1.46	0.25			1.5	0.19	1.58	0.4

LVDd: LV diameter in diastole, LVDs: LV diameter in systole, IVSd: septal thickness in diastole, IVSs: septal thickness in systole, LVPWd: LV free wall thickness in diastole, LVPWs: LV free wall thickness in systole, FS%: left ventricular fractional shortening, %PWth: percentage left ventricular free wall thickening, EPSS: mitral valve E point to septal separation, Vcf: velocity of circumferential fibre shortening.

### 3.5.4 Dobermanns with DCM

Although there are a number of reports of M-mode values in asymptomatic Dobermanns that subsequently developed DCM (O'Grady and Horne, 1995b; O'Grady and Horne, 1995c), Calvert and Brown's study is the only one that lists values for Dobermanns already diagnosed with overt DCM (Calvert and Brown, 1986). A comparison with the values obtained in this study is listed in table 3.12.

On the whole, the Dobermanns in the present study appear to have had a greater degree of LV dilation, thinner LV walls, and worse FS%, Vcf and PWth% than the dogs in Calvert's study.

### 3.5.5 Comparison of normal dogs and dogs with DCM

All the variables showed highly significant differences in a one-way ANOVA or Kruskal-Wallis test between the three groups ( $p < 0.001$ ). Multiple pairwise comparisons using A Tukey test or Dunn's test showed significant differences for all the variables between the normal dogs and dogs with DCM.

More difficulties lie in distinguishing normal dogs from dogs with early, asymptomatic DCM. There appears to be an occult stage in dilated cardiomyopathy in both man and dogs, during which time myocardial function is deteriorating but the subject remains asymptomatic. There has been much interest in detecting these early changes noninvasively with echocardiography in human DCM, particularly when screening relatives of affected patients (Zachara *et al.*, 1993) (Michels *et al.*, 1992). However, the issue of defining normal M-mode values in dogs assumes particular importance in breeds with a predisposition for DCM. Based on many published criteria for the diagnosis of DCM based on M-mode, one would be suspicious about myocardial function in some of the GSH pointers in this study.

It is clear that the M-mode criteria for normality in Dobermanns are still incompletely defined. It is possible that the prevalence of DCM may be different in different populations of Dobermanns, so that the mean M-mode values in asymptomatic dogs will differ according to the prevalence of occult disease. This might explain the difference in normal values between the reports by Sottiaux and



Amberger (1997) and Calvert and Brown (1986) compared with the Canadian results. O'Grady and co-workers used a right parasternal long axis view to derive their M-mode values, although the method used by the other two groups was not reported. It is possible that differences may exist between M-modes derived from long-axis or short-axis, as higher values were found for FS% derived from M-mode versus from 2D images in human patients (Douglas *et al.*, 1987). The cut-off points used for FS% and LV dimensions to separate normal dogs from those with occult DCM should take into account the greater requirement for sensitivity, or specificity. Using a lower value as the cut-off point for FS% will result in improved specificity at the cost of sensitivity.

### 3.5.6 Comparison of Dobermanns and cocker spaniels

Dobermanns showed a significant difference from cockers for only four variables: LVDd, LVDd index, LVDs and aortic diameter. One might predict that the left ventricular diameters would be greater in the Dobermanns than the cockers purely on the basis of size, so these differences are not altogether surprising. More surprising is the difference in LVDd index, where the cockers had a greater degree of end-diastolic ventricular dilation. The LVDs index was also greater in cockers than Dobermanns, although this did not reach statistical significance. This greater degree of dilation in cockers probably reflects a different shape of the left ventricle compared with Dobermanns, as the end-diastolic volume index was not statistically different (see Chapter 4). The left ventricles of Dobermanns tend to be longer and less spherical than cockers, even when affected with DCM. A more spherically-shaped left ventricle has been associated with poorer survival times in human patients with

DCM (Douglas *et al.*, 1989), although this did not appear to be true for these cocker spaniels.

Neither was the degree of LV hypertrophy greater in the cocker spaniels than the Dobermanns, as reflected in similar values for LVDd/LVPWd. A greater degree of wall thickness relative to LV diameter has been associated with improved survival in human DCM patients (Benjamin *et al.*, 1981). Missri examined the reciprocal of this index (mean diastolic LV wall thickness/ LV diastolic diameter), and was able to separate short-term from long-term DCM survivors, with long-term survivors having a hypertrophy-dilation index  $>0.10$  (Missri, 1984). In this study, Dobermanns had an index of  $0.12 \pm 0.02$ , and cockers had an index of  $0.12 \pm 0.04$ .

The systolic ratio between LV diameter and wall thickness has been used as an index of wall stress without taking into account LV systolic pressure (Zoghbi *et al.*, 1987), and Minors and O'Grady (1998) found differences in this index between normal Dobermanns and dogs with occult DCM. In their study, normal Dobermanns had a mean ratio of LVDs/LVPWs of  $2.9 \pm 0.09$ , and Dobermanns with occult DCM had a ratio of  $3.85 \pm 0.25$ . The normal dogs in the present study had lower ratios ( $2.07 \pm 0.46$ ) than Minors and O'Grady's normal Dobermanns, and the Dobermanns with DCM had much higher ratios ( $8.84 \pm 1.75$ ) than their Dobermanns with occult DCM. Once again, there was no significant difference between the affected Dobermanns and cocker spaniels in this study, although the ratio was slightly lower in the cocker spaniels ( $8.29 \pm 2.03$ ).

There were no clear differences in systolic function between the two affected breeds. FS%, Vcf and EPSS were nearly identical (9.6 and 9.0%; 0.62 and 0.63 circs/s; and 1.8 and 2.0 cm in Dobermanns and cockers respectively. There was

a non-significant trend towards a higher mean %PWth in the cocker spaniels (17.9% versus 10.6%). Percentage free wall thickening was the only echocardiographic variable to be of any prognostic value in the study of 37 dogs with DCM by Monnet and co-workers (1995), but even this was not an independent prognostic indicator.

### 3.6 CONCLUSIONS

M-mode echocardiographic values were recorded in normal GSH pointers, and showed good reproducibility for all measurements. The values for systolic function (as assessed by FS% and Vcf ) were lower than commonly reported in conscious dogs, apart from those published for greyhounds. There was no supporting evidence that these values reflected a pathological state in these dogs, and development of breed-specific reference ranges for M-mode echocardiographic variables is to be encouraged.

The M-mode echocardiographic values reported here clearly distinguished the normal dogs from the dogs with DCM. Differences between normal dogs and dogs with DCM persisted after indexing the values to body surface area.

Although significant differences were found between affected Dobermanns and cocker spaniels for LVDd, LVDd index, LVDs and Ao, these differences did not account for the differences in survival, according to previous reports of useful M-mode prognostic indicators.

## CHAPTER 4: EVALUATION OF VENTRICULAR FUNCTION IN NORMAL DOGS AND DOGS WITH DCM USING TWO- DIMENSIONAL ECHOCARDIOGRAPHY

### 4.1 INTRODUCTION

Two-dimensional echocardiography (2DE) is the standard noninvasive technique for measuring left ventricular volume and function in human DCM, as it provides the best compromise between accuracy, practicality and availability (Dujardin *et al.*, 1997b; Oh *et al.*, 1999b). Nevertheless, there does not appear to have been widespread adoption of 2DE to calculate LV volumes in clinical canine cardiac disease according to the veterinary literature.

One of the reasons may be that there have been only a few reports of normal values (O'Grady *et al.*, 1986; Sisson *et al.*, 1989), and clinicians have felt more comfortable using the familiar and long-established M-mode dimensions and fractional shortening. Recent veterinary reviews of quantitative echocardiography have acknowledged that 2DE techniques offer improved accuracy in assessing global LV performance, without offering examples of application of 2DE (Boon, 1998; Kienle and Thomas, 1995).

### 4.2 AIMS

1. To measure 2DE values in a group of normal German short-haired pointers, and to establish the repeatability of these measures.

2. To compare the 2DE results obtained in the normal dogs with those from a group of Dobermanns with DCM, and a group of English cocker spaniels with DCM.
3. To compare the results obtained in the two groups of dogs with DCM, to identify any differences in chamber dimensions or systolic function that might merit further investigation as prognostic indicators in canine DCM.

## 4.3 BACKGROUND

### 4.3.1 2D echocardiographic assessment of human DCM

The main 2DE findings reported in DCM are of a more spherical, hypocontractile LV, with or without dilation of the other chambers (Feigenbaum, 1994; Levine, 1994; Oh *et al.*, 1999c). A reduction in LV ejection fraction (EF%) is a universal finding, with the diagnosis requiring an ejection fraction less than two SD below the LV ejection fraction of normal individuals (Melton *et al.*, 1996). In practice, this is often taken to be <50% (Romeo *et al.*, 1989), although it may range from <45% (Diaz *et al.*, 1987) to <55% (Gavazzi *et al.*, 1993). The latter figure may overlap with some normal subjects, as one 2DE study in 1782 young healthy adults found 90% of individuals to have an ejection fraction between 53% and 71% (Wong *et al.*, 1995).

The increase in LV diameter relative to length is an often-quoted feature, which may be more marked than with other causes of LV dilation (D'Cruz *et al.*, 1989; Douglas *et al.*, 1989). Additional 2DE features include apical “tenting” of the mitral leaflets, shifting coaptation of the mitral valve more apically and possibly contributing to mitral regurgitation (Levine, 1994). Increased echogenicity of

intraventricular blood (LV “smoke”) may also be a feature of human DCM patients, and may represent increased risk of thromboembolic complications (Levine, 1994).

#### 4.3.2 2DE prognostic indicators in human DCM

Survival in human patients with DCM has been shown to correlate with ejection fraction in a number of studies (Diaz *et al.*, 1987; Gradman *et al.*, 1989; Likoff *et al.*, 1987; Rihal *et al.*, 1994; Romeo *et al.*, 1989; Unverferth *et al.*, 1984).

A number of studies of human dilated cardiomyopathy patients have shown low ejection fraction to be a predictor of poor outcome (Gavazzi *et al.*, 1993) (Dubois-Rande *et al.*, 1992) (Keogh *et al.*, 1988) (Anguita *et al.*, 1993) (Clements *et al.*, 1991) (Schwarz *et al.*, 1984) (Fruhwald *et al.*, 1996) (Unverferth *et al.*, 1984). Almost an equal number of studies have found that ejection fraction is not a predictor of survival in human DCM patients (Pelliccia *et al.*, 1994) (Kelly *et al.*, 1990) (Saxon *et al.*, 1993) (Abramson *et al.*, 1992) (Missri, 1984).

The probable reason for these conflicting results is that ejection fraction does appear to have a significant effect on prognosis when patients with only moderate impairment of systolic function are included in the study group. In most studies, patients with ejection fractions between 35-45% were found to have improved survival when compared with patients with ejection fractions <20%. In studies where only patients with severe systolic dysfunction are included (EF% <30%), there was less tendency for EF% to be correlated with survival. It appears that factors other than systolic dysfunction become more important in predicting survival once myocardial function reaches this level of hypocontractility.

Other 2DE estimates previously associated with prognostic value include a number of indices of chamber geometry, such as the “sphericity index” (Douglas *et al.*, 1989). LV volumes appear to have better prognostic power than LV linear dimensions (Diaz *et al.*, 1987; Juilliere *et al.*, 1988; Romeo *et al.*, 1989; Vecchia *et al.*, 1998). The presence of spontaneous contrast in the left atrium has been associated with poorer survival times (Shen *et al.*, 1996).

#### 4.3.3 2DE assessment of canine DCM

Use of quantitative 2D echocardiography in normal dogs is discussed in chapter 1. Minors and O’Grady (1998) measured left atrial area and circumference in Dobermanns with occult DCM in a recent report, although there was no significant difference in comparison with normal Dobermanns. There have been few reports of 2DE measurements in dogs with clinical DCM, apart from a report by Dukes McEwan (1998) of Newfoundlands with DCM that mentioned LV volumes and EF% (Dukes McEwan, 1998a). Mean EF% was  $24.1 \pm 9.15\%$  in affected dogs, compared with  $41.42 \pm 11.84\%$  in apparently healthy Newfoundlands.

### 4.4 MATERIALS AND METHODS

#### 4.4.1 Equipment

All 2DE studies were carried out using a Vingmed CFM 700 ultrasound system (Vingmed CFM 700, Dasonics, Sonotron, Bedford, UK) using either a 5.0 MHz or 2.25 MHz annular array transducer. The transducers were mechanically steered with dynamic focussing. The 5.0 MHz probe produced 2D images with a central bandwidth around 5.0 MHz and the 2.25 MHz probe had a central bandwidth

around 2.25MHz. A simultaneous ECG was recorded in all studies (limb lead II) using pre-gelled adhesive electrodes attached to the main pads of the feet, or to the limbs above the carpus and tarsus (F60, Skintact ECG pads, HA West Ltd, 41 Watson Crescent, Edinburgh, UK). Electrodes were further secured using permeable non-woven synthetic adhesive tape (Micropore, 3M Health Care Ltd, 3M House, Morley St, Loughborough, Leics, UK). Studies were recorded onto VHS videotape by a Panasonic AG 6200 video cassette recorder. Still transparencies could be recorded onto 35 mm film using a freeze frame recorder (Polaroid UK Limited, Ashley Road, St Albans, Herts, UK).

#### *4.4.1.1 Echocardiograph settings*

The depth setting was adjusted to maximise the size of the cardiac image within the sector. The required depth would vary between 10 to 12 cm for the German shorthaired pointers, 8 to 12 cm for the cocker spaniels, and 10 to 14 cm for the Dobermanns. The sector angle was generally 90°, which yielded a frame rate of 26 frames/second at these depth settings during 2DE. Post-processing controls were pre-set, so that reject and compression levels were the same for each study. The time gain compensation controls were adjusted as an even 'ramp' up to the maximum image depth. Overall gain settings were adjusted for each animal to produce the optimal image quality. 2D imaging was generally carried out using the 5 MHz transducer.



#### 4.4.2 Methods

Dogs were scanned unsedated, and manually restrained in right lateral recumbency on a purpose-built table. The table consisted of a metal base with a removable rigid perspex top, with had a V-shaped section removed halfway along the edge of one of the long sides. This was to facilitate access for the transducer to the dependent part of the thorax, so that right parasternal views were obtained with the dog lying in right lateral recumbency.

2DE images were derived from a 2D right parasternal long axis image, optimised to produce the largest LV dimension without inclusion of papillary muscles (Thomas *et al.*, 1993). This was usually a pure LV inlet view, without any of the LV outflow tract visible. By moving the transducer cranially one intercostal space, a view optimised for the left atrium could generally be obtained. This view included the mitral valve, interatrial septum and right pulmonary vein. By rotating the transducer 90° anti-clockwise and angling dorsally and cranially, a short-axis view of the heart base was obtained, including the aortic valve in short axis, and the left atrium and left auricular appendage. These images were recorded on videotape, and measured off-line.

#### 4.4.3 Protocol

##### *4.4.3.1 Normal GSH pointers, reproducibility studies*

All dogs were clinically normal on physical examination, and had no history that would suggest cardiac disease. The full details of these dogs are described in chapter 2. Eleven dogs were scanned on one occasion only, and two dogs were scanned on each of five successive days.

#### 4.4.3.2 Dogs with DCM

All dogs had been presented to the R(D)SVS for diagnosis and/or treatment of dilated cardiomyopathy. A diagnosis of DCM was based on the presence of congestive heart failure with a dilated hypocontractile heart and an absence of congenital, valvular or pericardial abnormalities. The selection criteria are described in more detail in Chapter 2.

#### 4.4.4 2DE measurements

Measurements were made off-line from the recorded video cassette tapes. The Vingmed system integral measuring software was accessed by entering a calibration code recorded in the lower left corner of the recorded image when prompted. The measurement menu available for 2DE image analysis allowed measurement of LV length and area, left atrial and aortic dimensions in diastole and systole using electronic calipers positioned by means of a tracker ball. End-diastolic frames were chosen as the frame in which mitral valve closure began, and end-systolic frames were chosen as the last frame before mitral valve opening, in accordance with ASE recommendations (Schiller *et al.*, 1989). The left ventricular length was measured from the midpoint of a line across the mitral valve annulus, to the endocardial border at the apex (figure 4.1). The inner endocardial borders of the left ventricle were traced using a trackerball, excluding the papillary muscles.

The following variables were measured: LV diastolic area, LV diastolic length, LV systolic area, LV systolic length, LA diameter at the widest point, LA

diameter just proximal to the level of the mitral annulus, aortic diameter in short axis, and LA diameter in short axis.

Two formulae were used to calculate LV volumes: a modified Simpson's technique, which divided the planimetered long-axis area into 20 equal sections, using the following formula, where A is the LV long axis area, and L is the LV maximal length:

Formula 4.1

$$\frac{\pi}{4} \sum_{i=1}^{20} A^2 \cdot \frac{L}{20}$$

The area-length formula was also based on the same long-axis length (L) and planimetered area (A):

Formula 4.2

$$\frac{8(A)^2}{3\pi L}$$

Stroke volume (SV) was calculated from the derived LV volumes, end-diastolic volume (EDV) and end-systolic volume (ESV):

Formula 4.3

$$SV = EDV - ESV$$

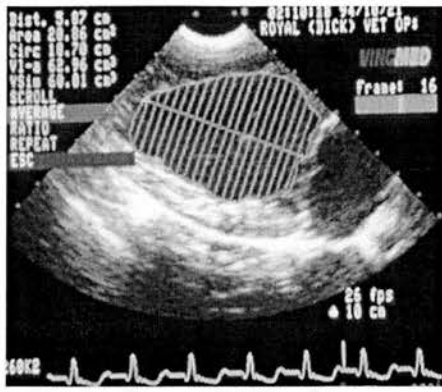
Ejection fraction (EF%) was calculated using the following formula:

Formula 4.4

$$EF\% = \frac{EDV - ESV}{EDV} \times 100$$

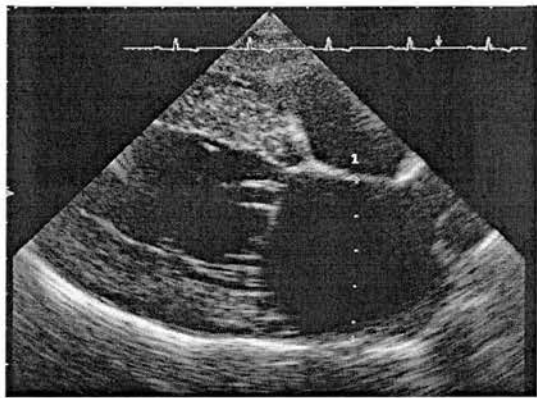
Two left atrial diameters were measured from the right parasternal long axis view: one at the maximum width, parallel with the mitral annulus and bisecting the atrium (LA diameter, figure 4.2), and the width at the mitral annulus itself (LA annular diameter). The left atrial area was also traced in this view, excluding the left pulmonary vein. In the short axis view, measurements were made of the aortic diameter in diastole, and of the left atrial diameter at the same level, and the same frame (figure 4.3) (Haggstrom *et al.*, 1994).

Figure 4.1: Two-dimensional echocardiogram showing left ventricular volume measurement.



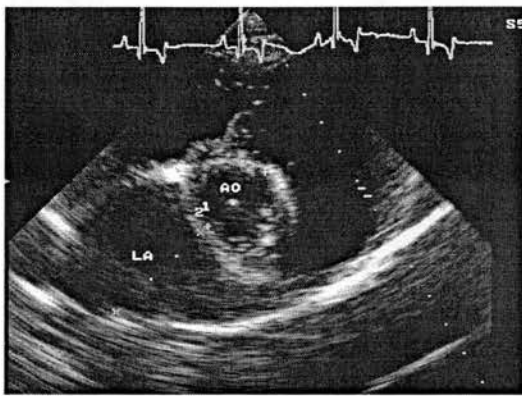
Right parasternal long axis view, showing tracing of left ventricular endocardial border, and application of a modified Simpson's rule to calculate left ventricular volume.

Figure 4.2: Measurement of the left atrium (long axis)



Measurement of the left atrium in a right parasternal long axis view. The diameter of the left atrium is measured parallel to the mitral annulus, at the widest point of the left atrium.

Figure 4.3: Measurement of the left atrium (short axis)



The aortic diameter (ao) is measured from the midpoint of the right coronary cusp to the commissure of the left and non-coronary cusps, and the left atrial diameter (LA) is measured as an extrapolation of this point.

#### 4.4.5 Statistical methods

All values are expressed as mean  $\pm$  SD. Coefficients of variation were calculated for the repeated measures in the two normal GSH pointers by dividing the standard deviation by the mean, and multiplying by 100 to express the result as a percentage. The values were compared between GSH pointers, Dobermanns and cocker spaniels by one way analysis of variance when data were normally distributed with equal variance, and by Kruskal-Wallis one way analysis of variance when a test for normality or equal variances failed. When a significant difference was found between groups, multiple pair-wise comparisons were made using a Tukey test for normally distributed data, or Dunn's method following a Kruskal-Wallis test. The significance level was set at  $p < 0.05$ . All statistical analyses were carried out using a proprietary statistical software programme (SigmaStat 2.0, Jandel Scientific, San Rafael, CA, USA). Graphs were plotted using SigmaPlot (Jandel Scientific, San Rafael, CA, USA).

### 4.5 RESULTS

#### 4.5.1 German short-haired pointers

The 2DE area and length measurements for the individual GSH pointers are presented in table 4.1, with left atrial and aortic measurements in table 4.2, and LV volume measurements using a modified Simpson's rule in table 4.3. Volume measurements calculated using an area-length formula are listed in Appendix 2. Recordings were considered of adequate quality for measuring in all dogs in which the required views were recorded. The short axis left atrium and aorta were not recorded in dogs 5, 7, 8, 9, and 10, nor the long axis left atrium in dogs 9 and 10, due

to failure to record sufficient cardiac cycles rather than technical difficulties in acquiring appropriate quality images.

Volume calculations using the area-length consistently resulted in greater values than the modified Simpson's calculations, with a mean diastolic volume 11.4% higher than the Simpson's equivalent ( $p < 0.001$ ), and 10.2% higher for systolic volumes ( $p < 0.001$ ). The modified Simpson's method yielded a mean EDV of  $72.43 \pm 13.47$  mL, an ESV of  $28.46 \pm 7.39$  mL, and an EF% of  $61.06 \pm 4.29\%$ . Mean SV was  $43.96 \pm 7.22$  mL.

The LA diameter was consistently larger than the LA annular diameter ( $4.08 \pm 0.20$ , versus  $3.56 \pm 0.24$ ). The mean 2DE LA:aorta ratio obtained from the short-axis view was  $1.12 \pm 0.11$ , which was not significantly different from  $1.05 \pm 0.08$ , obtained with M-mode in the normal dogs (using a paired t-test).

Table 4.1: 2DE LV area and length measurements in individual normal GSH pointers.

Dog	LV diastolic area cm <sup>2</sup>	LV diastolic length cm	LV systolic area cm <sup>2</sup>	LV systolic length cm	LV length/ diameter (diastole)	LV length/ diameter (systole)
1	25.4	6.49	15.68	5.63	1.59	1.85
2	29.84	7.35	18.59	6.30	1.62	1.75
3	30.58	7.95	16.85	6.28	1.75	1.77
4	28.25	7.47	15.92	6.07	1.48	1.65
5	27.89	7.25	15.26	5.91	1.73	1.98
6	26.98	7.43	14.37	6.26	1.87	*2.42
7	21.22	6.09	11.48	4.86	1.51	1.75
8	18.83	6.22	10.17	5.04	1.74	2.07
9	21.37	6.4	11.80	5.16	1.55	1.86
10	22.76	6.47	12.07	5.29	1.49	1.73
11	25.81	6.83	16.13	6.22	1.76	2.05
12	25.1	7.5	12.63	6.13	1.87	2.25
13	26.77	7.71	14.23	5.89	*2.07	2.16
mean	25.45	7.01	14.24	5.77	1.69	1.95
SD	3.53	0.62	2.46	0.52	0.18	0.23

\*Indicates value >2SD from the mean. 2DE: 2-dimensional echocardiography, LV: left ventricle, GSH: German short-haired, SD: standard deviation. Note that LV diameter was recorded from M-mode studies.



Table 4.2: 2DE left atrial and aortic measurements in individual normal GSH pointers.

Dog	Lax LA area cm <sup>2</sup>	Lax LA area index cm <sup>2</sup> /m <sup>2</sup>	LA Lax diameter cm	LA Lax diameter index cm/m <sup>2</sup>	LA Lax annular diameter cm	Short- axis aorta cm	Short- axis LA cm	LA: Ao
1	10.61	12.06	4.03	4.58	3.44	2.32	2.97	1.28
2	10.61	11.79	4.26	4.73	3.57	2.13	2.01	0.94
3	12.29	12.17	4.36	4.32	4.04	2.42	2.66	1.10
4	9.63	*9.53	4.05	4.01	3.52	*2.78	2.99	1.08
5	11.00	12.50	4.01	4.56	3.79	NR	NR	NR
6	10.81	13.86	4.11	5.27	3.72	2.12	2.15	1.01
7	9.89	11.92	4.08	4.92	3.34	NR	NR	NR
8	9.62	11.32	3.87	4.55	3.16	NR	NR	NR
9	NR	NR	NR	NR	NR	NR	NR	NR
10	NR	NR	NR	NR	NR	NR	NR	NR
11	11.09	13.69	4.13	5.10	3.70	2.29	2.51	1.10
12	8.88	10.96	3.68	4.54	3.40	2.21	2.65	1.20
13	11.01	12.51	4.35	4.94	3.46	2.10	2.57	1.22
mean	10.49	12.03	4.08	4.68	3.56	2.30	2.56	1.12
SD	0.93	1.21	0.20	0.36	0.24	0.23	0.35	0.11

\*Indicates value >2SD from the mean. 2DE: 2-dimensional echocardiography, LA: left atrium, GSH: German short-haired, Lax: long axis, index: indicates value was normalised for body surface area, SD: standard deviation, NR: not recorded.

Table 4.3: 2DE LV volume measurements in GSH pointers using Simpson's method.

Dog	EDV	EDVI	ESV	ESVI	SV	SV index	EF%
	mL	mL/m <sup>2</sup>	mL	mL/m <sup>2</sup>	mL	mL/m <sup>2</sup>	%
1	75.03	85.26	34.08	38.73	40.95	46.53	54.58
2	93.04	103.38	42.65	47.39	50.39	55.99	54.16
3	93.59	92.66	37.90	37.52	55.69	55.14	59.50
4	82.49	81.67	32.14	31.82	50.35	49.85	61.04
5	80.37	91.33	30.61	34.78	49.76	56.55	61.91
6	74.85	95.96	26.36	33.79	48.49	62.17	64.78
7	56.08	67.57	21.94	26.43	34.14	41.13	60.88
8	48.41	56.95	17.52	20.61	30.89	36.34	63.81
9	57.47	61.14	21.24	22.60	36.23	38.54	63.04
10	68.19	64.94	23.51	22.39	44.68	42.55	65.52
11	73.38	90.59	33.17	40.95	40.21	49.64	54.80
12	66.15	81.67	21.44	26.47	44.71	55.20	67.59
13	72.48	82.36	27.43	31.17	45.05	51.19	62.16
mean	72.43	81.19	28.46	31.90	43.96	49.29	61.06
SD	13.47	14.41	7.39	8.04	7.22	7.85	4.29

GSH: German Short-Haired, EDV: left ventricular end-diastolic volume, EDVI: EDV normalised for body surface area, ESV: left ventricular end-systolic, ESVI: ESV normalised for body surface area, SV: left ventricular stroke volume, SV index: SV normalised for body surface area. EF%: left ventricular ejection fraction, SD: standard deviation.

#### 4.5.1.2 Reproducibility

The repeated measurements in two normal dogs are shown in tables 4.4 to 4.6. Coefficients of variation for the repeated measures varied from 1.5% for left ventricular diastolic length in dog 1, to 13.4% for LV end-systolic volume (both methods) in dog 1. The coefficient of variation also exceeded 10% for stroke volume in dog 2 (12.78% for the area-length method, and 10.5% for Simpson's). There were no other variables in which the coefficients of variation exceeded 10%. Reproducibility was lower for systolic measurements than for diastolic measurements. Coefficients of variation for EDV were 4.6% for dog 1, and 8.5%

for dog 2 with Simpson’s formula. Coefficients of variation for ESV (13.4% and 6.1%) and SV (5.5% and 10.5%) were higher than for ejection fraction (6.0% and 2.7%).

It was subjectively easier to find the landmarks for measuring left atrial diameter at the widest point than at the annulus. The coefficients of variation supported this, with values for the left atrial diameter measured at the widest point of 4.9% and 2.7%, compared with values at the annulus of 5.8% and 6.1%.

The reproducibility of the 2DE measurements was at least as good as for the M-mode variables (median CV% for all M-mode variables = 6.8%, median CV% for all 2DE variables = 5.5%), with no significant difference between the values for CV% for each method.

Table 4.4. Repeated 2DE LV area, length values for normal GSH pointers.

	D. area (cm <sup>2</sup> )		D. length (cm)		S. area (cm <sup>2</sup> )		S. length (cm)	
	Dog 1	Dog 2	Dog 1	Dog 2	Dog 1	Dog 2	Dog 1	Dog 2
Day 1	26.5	26.8	7.4	7.7	13.3	14.2	5.9	5.9
Day 2	25.1	27.9	7.5	8.3	12.6	15.9	6.1	6.9
Day 3	26.6	25.9	7.6	7.7	14.9	14.5	6.3	6.4
Day 4	26.8	23.7	7.7	8.3	14.7	14.3	6.4	6.7
Day 5	24.1	27.5	7.6	8.4	15.2	14.9	6.3	6.8
mean	25.8	26.4	7.6	8.1	14.2	14.8	6.2	6.6
SD	1.2	1.7	0.1	0.3	1.1	0.7	0.2	0.4
CV%	4.5	6.3	1.5	3.9	7.8	4.6	3.3	6.3

2DE: 2 dimensional echocardiography, LV: left ventricle, GSH: German short-haired, D.: diastolic, S.: systolic, SD: standard deviation, CV%: coefficient of variation.

Table 4.5. Repeated 2DE LV values for normal GSH pointers (Simpson's method).

	EDV (mL)		ESV (mL)		SV (mL)		EF (%)	
	Dog 1	Dog 2	Dog 1	Dog 2	Dog 1	Dog 2	Dog 1	Dog 2
Day 1	72.4	72.5	24.7	27.4	47.7	45.1	65.9	62.2
Day 2	66.2	72.8	21.4	28.9	44.7	44.0	67.6	60.4
Day 3	75.2	70.4	29.2	26.8	46.0	43.6	61.2	62.0
Day 4	72.7	58.6	24.6	24.3	48.0	34.3	66.1	58.5
Day 5	71.5	70.5	29.6	26.5	41.8	44.0	58.5	62.5
mean	71.6	69.0	25.9	26.8	45.7	42.2	63.9	61.1
SD	3.3	5.9	3.5	1.6	2.5	4.4	3.8	1.7
CV%	4.6	8.5	13.4	6.1	5.5	10.5	6.0	2.72

2DE: 2 dimensional echocardiography, LV: left ventricle, GSH: German short-haired, EDV: left ventricular end-diastolic volume, ESV: left ventricular end-systolic volume, SV: left ventricular stroke volume, EF: left ventricular ejection fraction, SD: standard deviation, CV%: coefficient of variation.

Table 4.6: Repeated 2DE left atrial and aortic values for normal GSH pointers

	LA area (cm)		LA diam-1 (cm)		LA diam-2 (cm)		Sax Ao (cm)		Sax LA (cm)	
	Dog 1	Dog 2	Dog 1	Dog 2	Dog 1	Dog 2	Dog 1	Dog 2	Dog 1	Dog 2
Day 1	9.43	11.01	4.2	4.4	3.2	3.5	2.37	2.10	2.61	2.57
Day 2	8.88	10.77	3.7	4.4	3.4	3.7	2.21	2.27	2.65	2.52
Day 3	11.17	11.54	4.0	4.1	3.8	4.1	2.32	2.27	2.92	3.06
Day 4	10.15	11.33	4.0	4.1	3.4	3.8	2.30	2.37	2.62	2.75
Day 5	10.16	11.50	3.9	4.3	3.5	3.6	2.43	2.40	2.59	2.95
mean	9.96	11.23	4.0	4.2	3.5	3.7	2.33	2.28	2.68	2.77
SD	0.86	0.33	0.2	0.1	0.2	0.2	0.08	0.12	0.14	0.23
CV%	8.68	3.0	4.9	2.7	5.8	6.08	3.53	5.14	5.12	8.45

2DE: 2 dimensional echocardiography, GSH: German short-haired, LA: left atrium, Ao: aorta, LA diam-1: left atrial diameter at widest point, LA diam-2: left atrial diameter just proximal to mitral annulus, Sax: short axis, SD: standard deviation, CV%: coefficient of variation.

#### 4.5.2 Dobermanns with DCM

The 2DE area and length measurements for Dobermanns with DCM are displayed in tables 4.7 to 4.9. Left ventricular volume measurements calculated by a modified Simpson's method are listed in table 4.9, and by an area-length method in Appendix 2. Adequate recordings were obtained in all dogs except Dobermann 5, where it was not possible to trace the entire LV area as a result of a change in the measurement software, causing a calculation window to obscure part of the LV.

According to the modified Simpson's method, mean EDV was  $139.81 \pm 31.12$  mL, ESV was  $109.41 \pm 27.11$  mL, and mean EF% was  $22.0 \pm 5.28\%$ . Mean SV was  $30.4 \pm 8.94$  mL. The mean LA diameter was  $6.11 \pm 0.59$  cm, and the mean LA area was  $27.58 \pm 5.8$  cm<sup>2</sup>.

Dobermann 1 had evidence of spontaneous echo-contrast in the left ventricle (figure 4.3).

Table 4.7: 2DE LV length / area measurements in Dobermanns with DCM.

Dog	LV diastolic area	LV diastolic length	LV systolic area	LV systolic length	LV length/ diameter (diastole)	LV length/ diameter (systole)
	cm <sup>2</sup>	cm	cm <sup>2</sup>	cm		
1	37.31	7.03	30.74	6.37	1.01	0.98
2	29.13	6.02	23.85	5.63	1.05	1.12
3	27.67	5.9	24.33	5.99	1.05	1.20
4	27.24	6.07	22.06	5.3	0.88	0.85
5	NR	NR	NR	NR	NR	NR
6	43.22	7.84	36.07	7.15	1.15	1.15
7	41.38	8.33	37.27	7.92	1.36	1.38
8	41.85	7.86	35.32	7.24	1.21	1.22
9	32.47	6.7	26.24	5.88	0.93	0.91
10	33.49	6.46	29.58	6.31	0.96	1.02
11	44.01	8.95	39.94	8.87	1.58	1.64
mean	35.78	7.12	30.54	6.67	1.12	1.15
SD	6.61	1.07	6.34	1.12	0.22	0.23

2DE: 2-dimensional echocardiography, LV: left ventricle, DCM: dilated cardiomyopathy, SD: standard deviation, NR: not recorded. Note that LV diameter was recorded from M-mode studies.

Table 4.8: 2DE left atrial and aortic measurements in Dobermanns with DCM.

Dog	Lax LA area cm <sup>2</sup>	Lax LA area index cm <sup>2</sup> /m <sup>2</sup>	LA Lax diameter cm	LA LAX diameter index cm/m <sup>2</sup>
1	22.58	22.14	5.76	5.65
2	26.42	27.52	6.48	6.75
3	25.17	26.21	5.58	5.81
4	18.72	*13.37	5.18	3.70
5	34.53	26.97	6.84	5.34
6	30.78	27.48	6.65	5.94
7	22.48	21.82	5.62	5.46
8	24.14	22.77	5.79	5.46
9	27.37	27.93	6	6.12
10	34.65	27.07	7	5.47
11	36.5	31.20	6.35	5.43
mean	27.58	24.95	6.11	5.56
SD	5.80	4.77	0.59	0.74

\*Indicates value >2SD from the mean. 2DE: 2-dimensional echocardiography, DCM: dilated cardiomyopathy, LA: left atrium, Lax: long axis, index: indicates value was normalised for body surface area, SD: standard deviation.

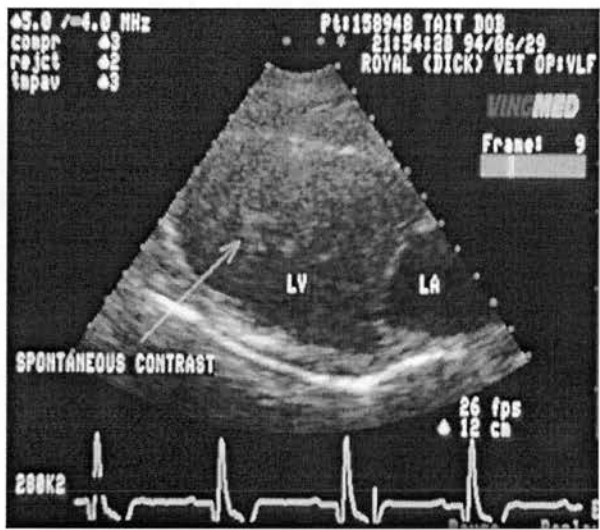
Table 4.9: 2DE LV volume measurements in Dobermanns with DCM

Dog	EDV	EDVI	ESV	ESVI	SV	SVI	EF%
	mL	mL/m <sup>2</sup>	mL	mL/m <sup>2</sup>	mL	mL/m <sup>2</sup>	%
1	155.29	152.00	115.76	113.00	39.53	38.75	25.50
2	108.60	113.00	81.55	84.94	27.05	28.18	24.90
3	99.34	103.00	81.03	84.40	18.31	19.07	18.40
4	96.48	68.90	71.44	51.00	25.04	17.89	25.90
5	NR	NR	NR	NR	NR	NR	NR
6	181.19	162.00	137.14	122.40	44.05	39.33	24.30
7	159.30	155.00	139.48	135.40	19.82	19.24	12.40
8	167.47	158.00	129.50	122.00	37.97	35.82	22.70
9	127.30	129.90	88.84	90.65	38.46	39.24	30.20
10	132.90	103.83	107.55	84.02	25.35	19.80	19.00
11	170.20	145.47	141.80	121.20	28.40	24.27	16.69
mean	139.81	129.11	109.41	100.90	30.40	28.16	22.00
SD	31.12	30.88	27.11	25.95	8.94	9.26	5.28

EDV: left ventricular end-diastolic volume, EDVI: EDV normalised for body surface area, ESV: left ventricular end-systolic, ESVI: ESV normalised for body surface area, SV: left ventricular stroke volume, SV index: SV normalised for body surface area. EF%: left ventricular ejection fraction, SD: standard deviation, NR: not recorded.

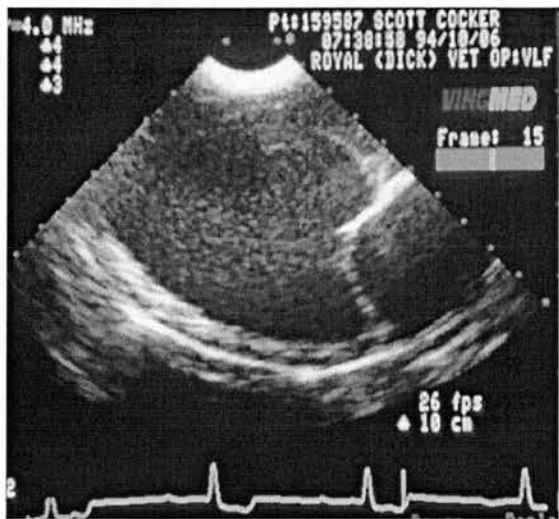


Figure 4.4: Two-dimensional echocardiogram in a Dobermann with dilated cardiomyopathy.



Right parasternal long axis view of Dobermann 1, showing a dilated left ventricle and spontaneous echo-contrast.

Figure 4.5: Two-dimensional echocardiogram of left atrium and left ventricle in a cocker spaniel with dilated cardiomyopathy.



Right parasternal long axis view in cocker 6, showing dilation of the left atrium and left ventricle.

### 4.5.3 Cocker Spaniels with DCM

The 2DE area and length measurements for cocker spaniels are listed in tables 4.10 to 4.12. Left ventricular volume measurements calculated by a modified Simpson's method are listed in table 4.12, and by an area-length method in Appendix 2. Adequate recordings were obtained in all dogs except Cocker 4, 10 and 11, where it was not possible to trace the entire LV area as a result of a change in the measurement software, causing a calculation window to obscure part of the LV. Long-axis left atrial measurements were not recorded in Cocker 8 for similar reasons.

According to the modified Simpson's method, mean EDV was  $90.26 \pm 25.17$  mL, ESV was  $72.4 \pm 21.13$  mL, and mean EF% was  $20.19 \pm 6.27\%$ . Mean SV was  $17.86 \pm 7.11$  mL. The mean LA diameter was  $4.47 \pm 0.72$  cm, and the mean LA area was  $12.51 \pm 3.2$  cm<sup>2</sup>. Short-axis LA and Ao measurements were made in 5 cockers, giving a mean 2DE LA:Ao ratio of  $1.72 \pm 0.30$ .

Table 4.10: 2DE area / length measurements in individual Cocker spaniels with DCM

Dog	LV diastolic area cm <sup>2</sup>	LV diastolic length cm	LV systolic area cm <sup>2</sup>	LV systolic length cm	LV length/ diameter (diastole)	LV length/ diameter (systole)
1	22.64	5.27	20.70	5.10	0.96	1.00
2	27.7	5.75	23.02	5.27	0.91	1.00
3	26.81	5.65	24.60	5.46	0.97	1.05
4	NR	NR	NR	NR	NR	NR
5	15.34	4.53	12.81	4.22	1.19	1.23
6	24.49	5.13	20.37	4.59	0.99	0.92
7	33.87	7.2	29.74	6.81	1.35	1.41
8	27.16	6.6	23.81	6.05	1.27	1.29
9	30.97	6.95	25.60	6.51	1.31	1.33
10	NR	NR	NR	NR	NR	NR
11	NR	NR	NR	NR	NR	NR
mean	26.62	5.89	22.58	5.50	1.12	1.15
SD	5.84	0.94	4.93	0.90	0.18	0.18

2DE: 2-dimensional echocardiography, LV: left ventricle, DCM: dilated cardiomyopathy, SD: standard deviation, NR: not recorded. Note that LV diameter was recorded from M-mode studies.

Table 4.11: 2DE left atrial and aortic measurements in cocker spaniels with DCM.

Dog	Lax LA area cm <sup>2</sup>	Lax LA area index cm <sup>2</sup> /m <sup>2</sup>	LA Lax diameter cm	LA LAX diameter index cm/m <sup>2</sup>
1	11.17	18.93	4.51	7.64
2	*19.86	*38.19	*5.98	*11.50
3	13.56	21.52	4.77	7.57
4	14.18	23.63	4.8	8.00
5	7.66	11.96	3.46	5.41
6	12.24	17.24	3.96	5.58
7	13.5	22.50	4.4	7.33
8	NR	NR	NR	NR
9	13.16	21.23	4.21	6.79
10	9.18	15.83	3.69	6.36
11	10.6	17.67	4.88	8.13
mean	12.51	20.87	4.47	7.43
SD	3.32	7.01	0.72	1.72

\*Indicates value >2SD from the mean. 2DE: 2-dimensional echocardiography, DCM: dilated cardiomyopathy, LA: left atrium, Lax: long axis, index: indicates value was normalised for body surface area, NR: not recorded, SD: standard deviation.

Table 4.12: 2DE LV volume measurements in Cocker spaniels with DCM

Dog	EDV	EDVI	ESV	ESVI	SV	SVI	EF%
	mL	mL/m <sup>2</sup>	mL	mL/m <sup>2</sup>	mL	mL/m <sup>2</sup>	%
1	77.29	131.00	67.54	114.40	9.75	16.52	12.60
2	105.50	202.00	80.35	154.00	25.15	48.36	23.80
3	98.51	156.00	87.25	138.00	11.26	17.87	11.40
4	NR	NR	NR	NR	NR	NR	NR
5	*38.93	60.80	27.75	43.30	11.18	17.46	28.70
6	81.51	115.00	64.85	91.30	16.66	23.46	20.40
7	121.28	202.00	99.47	165.70	21.81	36.35	18.00
8	91.74	164.00	73.94	132.00	17.80	31.78	19.40
9	107.30	173.06	78.07	125.92	29.23	47.15	27.24
10	NR	NR	NR	NR	NR	NR	NR
11	NR	NR	NR	NR	NR	NR	NR
mean	90.26	150.48	72.40	120.58	17.86	29.87	20.19
SD	25.17	47.38	21.13	38.70	7.11	13.11	6.27

\*Indicates value >2SD from the mean. EDV: left ventricular end-diastolic volume, EDVI: EDV normalised for body surface area, ESV: left ventricular end-systolic, ESVI: ESV normalised for body surface area, SV: left ventricular stroke volume, SV index: SV normalised for body surface area. EF%: left ventricular ejection fraction, SD: standard deviation, NR: not recorded.

#### 4.5.4 Comparison of GSH pointers and dogs with DCM

A summary of the mean values for each group (normal GSH pointers, Dobermanns with DCM, and cocker spaniels with DCM) is displayed in table 4.13, with the results of comparisons between the groups. The raw data for the three groups for all variables are displayed in graphs 4.1 to 4.16.

Table 4.13: Group mean 2DE values for GSH pointers, Dobermanns and Cocker spaniels, with results of group comparisons.

Variable	units	GSPs		Dobermanns		Cocker spaniels		ANOVA	Significant differences		
		mean	SD	mean	SD	mean	SD	P value	Dob vs GSP	CSp vs GSP	Dob vs CSp
Diast area	cm <sup>2</sup>	25.45	3.53	35.78	6.61	26.12	5.59	<0.001	+	-	+
Diast length	cm	7.01	0.62	7.12	1.07	5.89	0.94	0.01	-	+	+
EDV	mL	72.43	13.47	139.81	31.12	90.26	25.17	<0.001	+	-	-
EDV index	mL/ m <sup>2</sup>	81.19	14.41	129.11	30.88	150.48	47.38	<0.001	+	+	-
Diast L:D		1.69	0.18	1.12	0.22	1.12	0.18	<0.001	+	+	-
Syst area	cm <sup>2</sup>	14.24	2.46	30.54	6.34	22.58	4.93	<0.001	+	+	-
Syst length	cm	5.77	0.52	6.67	1.12	5.50	0.90	<0.001	+	-	+
ESV	mL	28.46	7.39	109.41	27.11	72.40	21.13	0.015	+	+	-
ESV index	mL/ m <sup>2</sup>	31.90	8.04	100.90	25.95	120.58	38.70	<0.001	+	+	-
Syst L:D		1.95	0.23	1.15	0.23	1.15	0.18	<0.001	+	+	-
Stroke vol	mL	43.96	7.22	30.40	8.94	17.86	7.11	<0.001	+	+	+
SV index	mL/ m <sup>2</sup>	49.29	7.85	28.16	9.26	29.87	13.11	<0.001	+	+	-
EF	%	61.06	4.29	22.00	5.28	20.19	6.27	<0.001	+	+	-
LA area	cm <sup>2</sup>	10.49	0.93	27.58	5.80	12.51	3.32	<0.001	+	-	+
LA area index	cm/m <sup>2</sup>	12.03	1.21	24.95	4.77	20.87	7.01	<0.001	+	+	-
LAD	cm	4.08	0.20	6.11	0.59	4.47	0.72	<0.001	+	-	+
LAD index	cm/m <sup>2</sup>	4.68	0.36	5.56	0.74	7.43	1.72	<0.001	+	+	-

2DE: 2 dimensional echocardiography, GSH: German short-haired, GSP(s): German short-haired pointer(s), ANOVA: analysis of variance, SD: standard deviation of the mean, Dob: Dobermann, CSp: cocker spaniel, diast: diastolic, EDV: end-diastolic volume, index: indicates value is normalised for body surface area, L: LV length, D: LV diameter from M-mode recording, syst: systolic, ESV: end-systolic volume, vol: volume, SV: stroke volume, EF: ejection fraction, LA: left atrium, LAD: left atrial diameter. All LV volumes were calculated using a modified Simpson's method, and LV diameters were recorded from M-mode studies.

Differences between the three groups by one-way analysis of variance or Kruskal-Wallis one-way analysis of variance on ranks showed significant differences for all variables at  $p < 0.001$  (except for diastolic length,  $p = 0.01$ ; and Simpson's end-systolic volume,  $p = 0.015$ ). Left ventricular diastolic area was significantly greater in the Dobermanns ( $35.8 \pm 6.6 \text{ cm}^2$ ) compared with the normal pointers ( $25.5 \pm 3.5 \text{ cm}^2$ ) and the cockers ( $26.2 \pm 5.6 \text{ cm}^2$ ), although left ventricular diastolic length was significantly shorter in the cockers ( $5.9 \pm 0.94 \text{ cm}$ ) compared with the pointers ( $7.0 \pm 0.62 \text{ cm}$ ) and Dobermanns ( $7.1 \pm 1.1 \text{ cm}$ ). End-diastolic volume (Simpson's formula) in the cockers ( $90.3 \pm 25.2 \text{ mL}$ ) did not differ significantly from either of the other groups, although the Dobermanns had a significantly bigger volume ( $139.8 \pm 31.2 \text{ mL}$ ) than the pointers ( $72.4 \pm 13.5 \text{ mL}$ ). When these end-diastolic volumes were indexed to body surface area, the pointers had significantly smaller volume indexes ( $81.2 \pm 14.4 \text{ mL/m}^2$ ) than the DCM dogs, with no difference between the Dobermanns ( $129.1 \pm 30.9 \text{ mL/m}^2$ ) and cockers ( $150.5 \pm 47.4 \text{ mL/m}^2$ ). The difference in ventricular geometry between the groups is also evidenced by the significantly greater LV diastolic length : diameter ratio in the pointers ( $1.69 \pm 0.18$ ) compared with the Dobermanns ( $1.12 \pm 0.22$ ) and cockers ( $1.12 \pm 0.18$ ), indicating a more spherical ventricular shape in the DCM dogs.

The systolic variables showed a similar pattern. Systolic area was significantly smaller in the pointers ( $14.2 \pm 2.5 \text{ cm}^2$ ), with no difference between the Dobermanns ( $30.5 \pm 6.3 \text{ cm}^2$ ) and cockers ( $22.6 \pm 4.9 \text{ cm}^2$ ). Systolic length was greater in the Dobermanns ( $6.7 \pm 1.1 \text{ cm}$ ), with no difference between the pointers ( $5.8 \pm 0.5 \text{ cm}$ ) and cockers ( $5.5 \pm 0.9 \text{ cm}$ ). End-systolic volume was smaller in the

pointers ( $28.5 \pm 7.4$  mL), with no difference between the Dobermanns ( $109.4 \pm 27.1$  mL) and cockers ( $72.4 \pm 21.2$  mL). The pattern was unchanged when the volumes were normalised for body surface area, with pointers having a significantly smaller volume index ( $31.9 \pm 8.0$  mL/m<sup>2</sup>), and no differences between the Dobermanns ( $100.9 \pm 26.0$  mL/m<sup>2</sup>) and cockers ( $120.6 \pm 38.7$  mL/m<sup>2</sup>).

Stroke volume was different between all three groups, with pointers having the largest stroke volume ( $44.0 \pm 7.2$  mL), followed by the Dobermanns ( $30.4 \pm 8.9$  mL), and then the cockers ( $17.9 \pm 7.1$  mL). When stroke volume was normalised for body surface area, the pointers had greater values ( $49.3 \pm 7.9$  mL/m<sup>2</sup>), with no differences between the Dobermanns ( $28.2 \pm 9.3$  mL/m<sup>2</sup>) and cockers ( $29.9 \pm 13.1$  mL/m<sup>2</sup>). Ejection fraction was also greater in the pointers ( $61.1 \pm 4.3$  %), with no differences between the Dobermanns ( $22.0 \pm 5.3$  %) and cockers ( $20.2 \pm 6.3$  %).

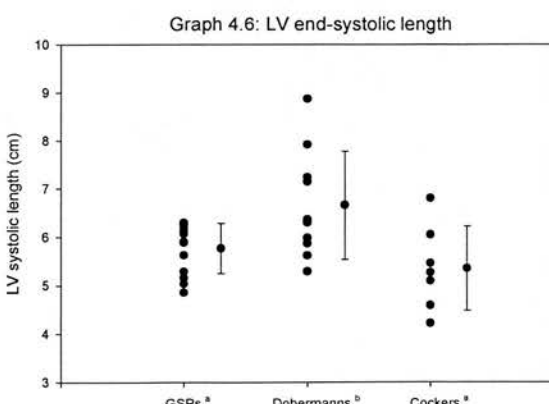
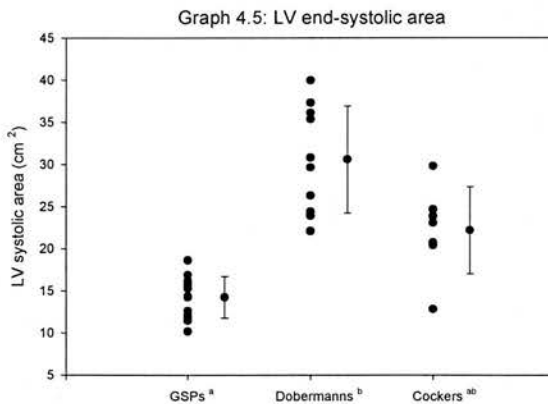
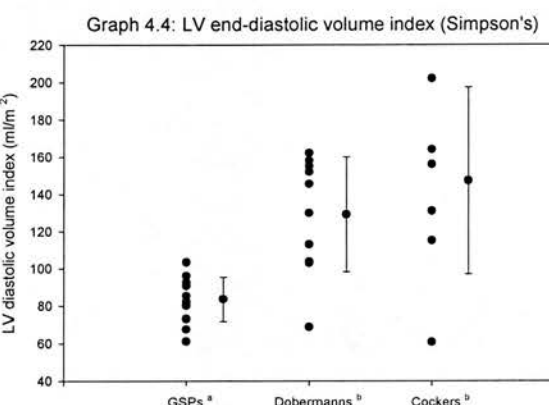
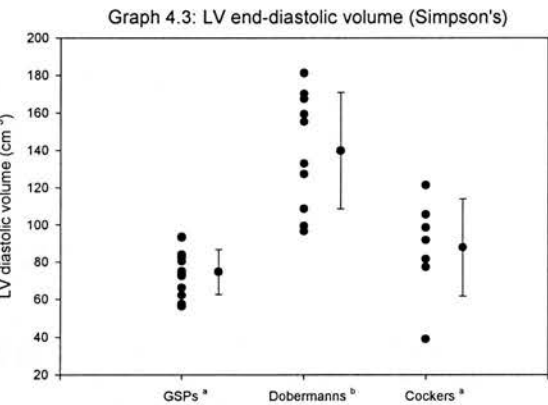
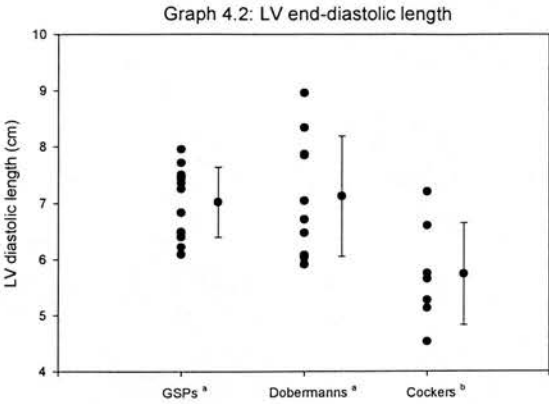
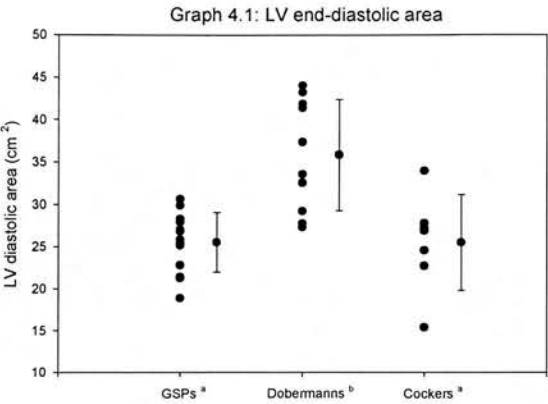
The Dobermanns had a significantly larger left atrial area compared with the other groups ( $27.6 \pm 5.8$  cm<sup>2</sup>), but there were no differences between the pointers ( $10.5 \pm 0.9$  cm<sup>2</sup>) and cockers ( $12.5 \pm 3.3$  cm<sup>2</sup>). When LA area was normalised to body surface area, the pointers had significantly smaller values ( $12.0 \pm 1.2$  cm<sup>2</sup>/m<sup>2</sup>), with no differences between the Dobermanns ( $25.0 \pm 4.8$  cm<sup>2</sup>/m<sup>2</sup>) and cockers ( $20.9 \pm 7.0$  cm<sup>2</sup>/m<sup>2</sup>). A similar pattern was seen with LA diameter, with the largest values in the Dobermanns ( $6.1 \pm 0.59$  cm) and no differences in the pointers ( $4.1 \pm 0.20$  cm) and cockers ( $4.5 \pm 0.72$  cm); and smallest values in LA diameter index in the pointers ( $4.7 \pm 0.36$  cm/m<sup>2</sup>), with no differences in the Dobermanns ( $5.6 \pm 0.7$  cm/m<sup>2</sup>) and cockers ( $7.4 \pm 1.7$  cm/m<sup>2</sup>). Although there was no significant difference in either LA area index or LA diameter index between the two DCM breeds, the



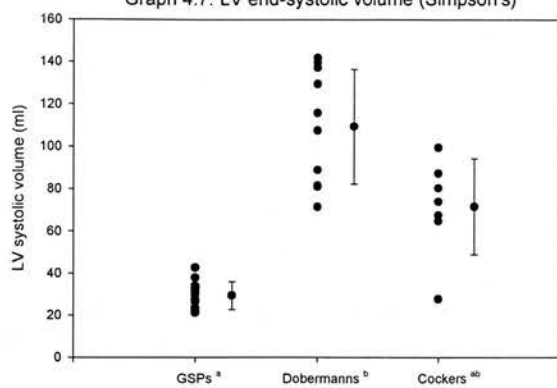
Dobermanns had a larger LA area index, whereas the cockers had a larger LA diameter index, suggesting a difference in LA shape.

When the 2DE LA:aorta values from the only three dogs with DCM that had this variable recorded were added to the values from the GSH pointers in the comparison between M-mode and 2DE LA:aorta, a significantly higher value was found with 2DE than with the same ratio recorded from M-mode ( $p < 0.05$ ), when compared using a paired t-test.

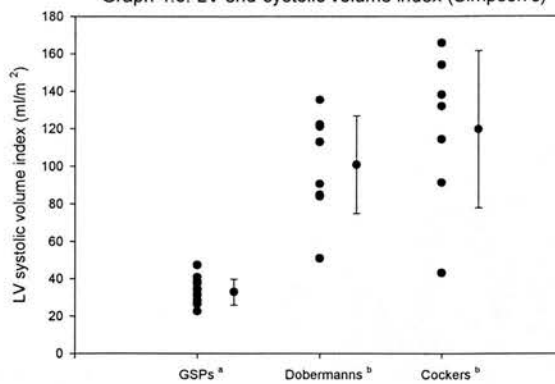
Comparison of 2DE variables in normal dogs and dogs with DCM.



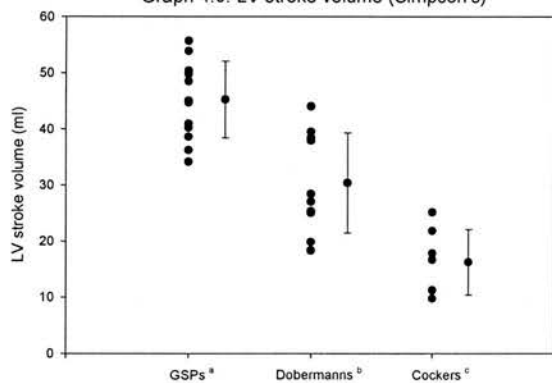
Graph 4.7: LV end-systolic volume (Simpson's)



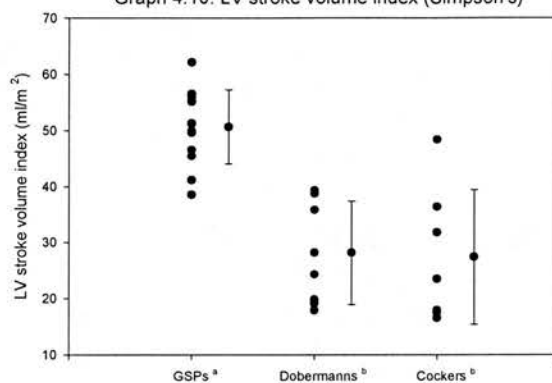
Graph 4.8: LV end-systolic volume index (Simpson's)



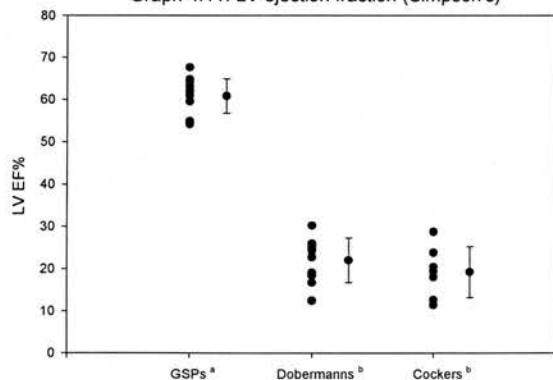
Graph 4.9: LV stroke volume (Simpson's)



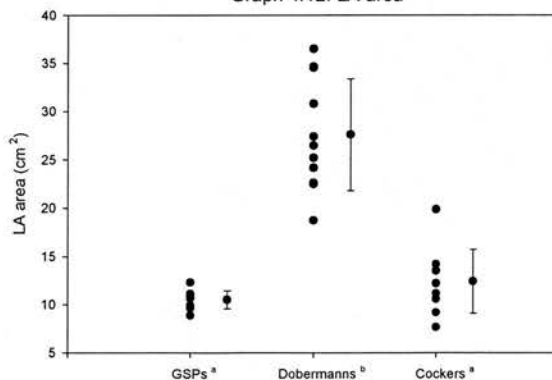
Graph 4.10: LV stroke volume index (Simpson's)

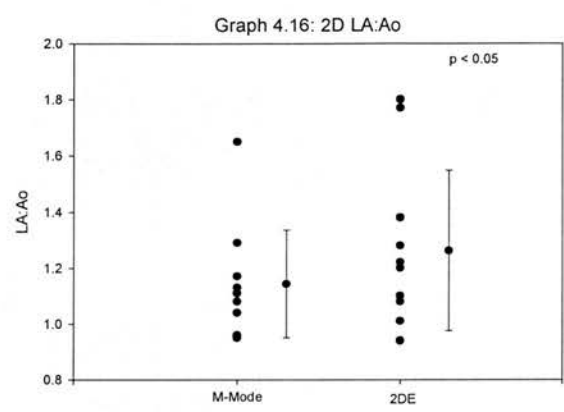
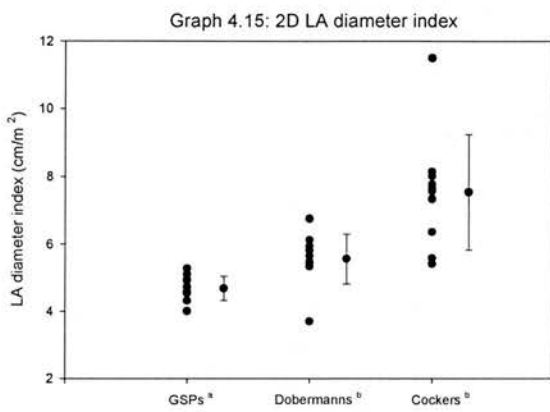
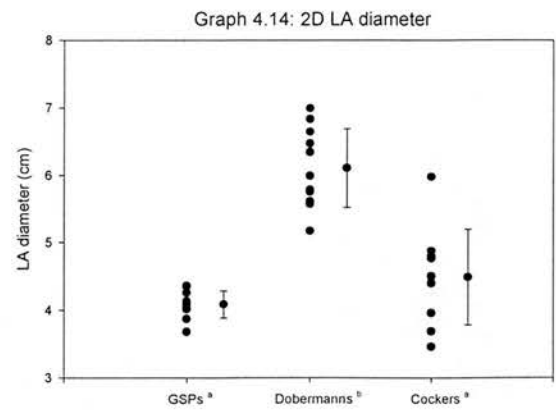
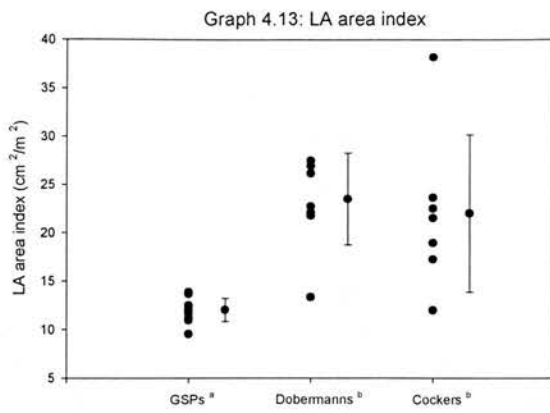


Graph 4.11: LV ejection fraction (Simpson's)



Graph 4.12: LA area





When German short-haired pointers (GSPs), Dobermanns or English cocker spaniels (cockers) were statistically different following analysis of variance, a different letter in superscript is ascribed (eg. GSP<sup>a</sup> and Dobermann<sup>b</sup> indicates the two groups were significantly different at the  $p < 0.05$  level in pairwise comparisons) . All volumes were calculated using a modified single-plane Simpson's method.

LV: left ventricle, GSPs: German short-haired pointers, LA: left atrium, Ao: aortic diameter.

## 4.6 DISCUSSION

The use of 2DE for the quantitative assessment of cardiac dimensions has been largely neglected in veterinary cardiology, despite becoming a standard technique for assessing ventricular function in human patients. The studies described in this chapter show that it is technically feasible to measure 2DE-derived LV volumes in both normal dogs and dogs with DCM, and that these measurements appear to be adequately reproducible when the studies are recorded and measured by a single observer.

The advances in the software packages incorporated into commercial echocardiography machines have greatly facilitated the calculation of LV volumes. The modified Simpson's calculation and the area-length formula used in this study were chosen because they were the formulae available on the Vingmed CFM 700. Fortuitously, these are also the two most widely recommended formulae (Schiller *et al.*, 1989). Most commercial echocardiography machines include at least an area-length algorithm for measurement of LV volumes, so that availability of suitable measurement software should not be a limiting factor for most veterinary echocardiographers.

### 4.6.1 Right parasternal long axis view and LV volumes in dogs

The volumes were measured from a right parasternal long axis view, which differs from the standard conventions used in measurement of human LV volumes (Schiller *et al.*, 1989). One of the arguments against using the right parasternal long axis view for LV volume measurements in human patients is the difficulty in

obtaining a complete image of the LV that includes the apex. This does not appear to be true in dogs, and there are numerous canine studies validating LV volume measurement, where it has been shown (at least in dogs under 30kg) that it is possible to obtain adequate images of the entire LV from the right parasternal long axis view (Feneley *et al.*, 1988; Schiller *et al.*, 1983; Sisson *et al.*, 1989; Stack *et al.*, 1987; Wyatt *et al.*, 1981). All of the Dobermanns in this study weighed 30 kg or greater, yet it proved possible to obtain an adequate window for visualisation of the entire LV from a right parasternal view in all dogs in which it was attempted. On radiographs, Dobermanns are generally perceived to have “narrow, upright” hearts, with an increased length to diameter ratio, so that one might predict that it would be particularly difficult to image the entire length of the ventricle from a parasternal window, compared to other breeds (Suter, 1984). The Dobermanns with DCM in this study had the greatest values for LV length, although they were not significantly different from the normal dogs in diastole. While O’Grady and co-workers (1986) found the greatest LV length in their right parasternal views rather than the apical ones, the maximum parasternal lengths was actually obtained from a view that included part of the LV outflow tract (O’Grady *et al.*, 1986). The parasternal views used in this study were pure LV inlet views, and it is possible that larger (and more accurate) volumes might have been generated from views that contained more of the LV outflow tract.

A recent prospective study suggested that the biplane Simpson’s method is superior to single area-length calculations in human post-infarct patients, as the latter tends to over-estimate volumes (St John Sutton *et al.*, 1998). However, the authors still recommended use of the single plane method, because of the difficulties in

obtaining two satisfactory views necessary for the biplane Simpson's method. The area-length method resulted in significantly higher volumes in this study, and the modified Simpson's method was chosen for all subsequent analyses. A single-plane Simpson's method appears to offer the best compromise between ease and accuracy in dogs, as regional wall motion abnormalities are less likely to be an issue than in human patients. Further studies are needed in closed chest dogs to compare volumes from right parasternal views (both inlet views, and views containing part of the LV outflow tract) with apical four-chamber and two-chamber views.

#### 4.6.2 Comparison of 2DE and M-mode values

##### *4.6.2.1 Reproducibility*

The reproducibility of the 2DE measurements was comparable to that for the M-mode variables. Translational motion is less likely to be an issue with measurements made from a 2D image, and it is easier to recognise when an adequate view is being recorded than with M-mode. In a study of 2DE reproducibility in normal humans, Himelman and others (1988) found mean inter-measurer and inter-technician variability (15% and 11% respectively) to be similar to that with angiography (3 - 15% on a beat-to-beat basis) and radionuclide studies (10 – 37% in sequential studies) (Himelman *et al.*, 1988). Image quality did vary between subjects, and this affected reproducibility. This may also be a factor in some dogs, but did not appear to be a problem in this study. On the basis of this study, technical difficulty and repeatability should not make 2DE measurements any more problematic than M-mode measurements in dogs.

#### *4.6.2.2 Normal reference intervals*

One of the reasons for the reluctance to embrace 2DE measurements may be the absence of normal reference intervals. This is an area of continuing controversy even with M-mode values, where current opinion suggests normal reference values may be required on an individual breed basis. LV volume measurements can be indexed to body surface area, and this may simplify interpretation. However, even this may be insufficient for the wide range of body size and shape seen with the spectrum of different canine breeds.

Kittleson and others (1984) used endsystolic volume index as a means of classifying myocardial failure in dogs with mitral regurgitation, using values based on human patients (Borow *et al.*, 1980). According to the results in this study, the mean GSH pointer value for ESVI fell outside the normal values used by Kittleson and others (1984), and Boon (Boon, 1998a). The relationship between ESVI and bodyweight is not likely to be constant over a range of bodyweights, and great care should be taken in extrapolating from human guidelines, or even normal values obtained in different breeds of dog until this relationship has been defined.

#### *4.6.2.3 Assessment of LA size*

In addition to LV volumes, the assessment of LA size can clearly be greatly facilitated by 2DE. There have been persistent problems with the measurement of LA size using M-mode echocardiography, because of the difficulty in crossing the body of the LA with the M-mode cursor. One commercially available echocardiography machine has in-built software that allows off-axis M-mode imaging (General Electric Vingmed System V), which can overcome this limitation.

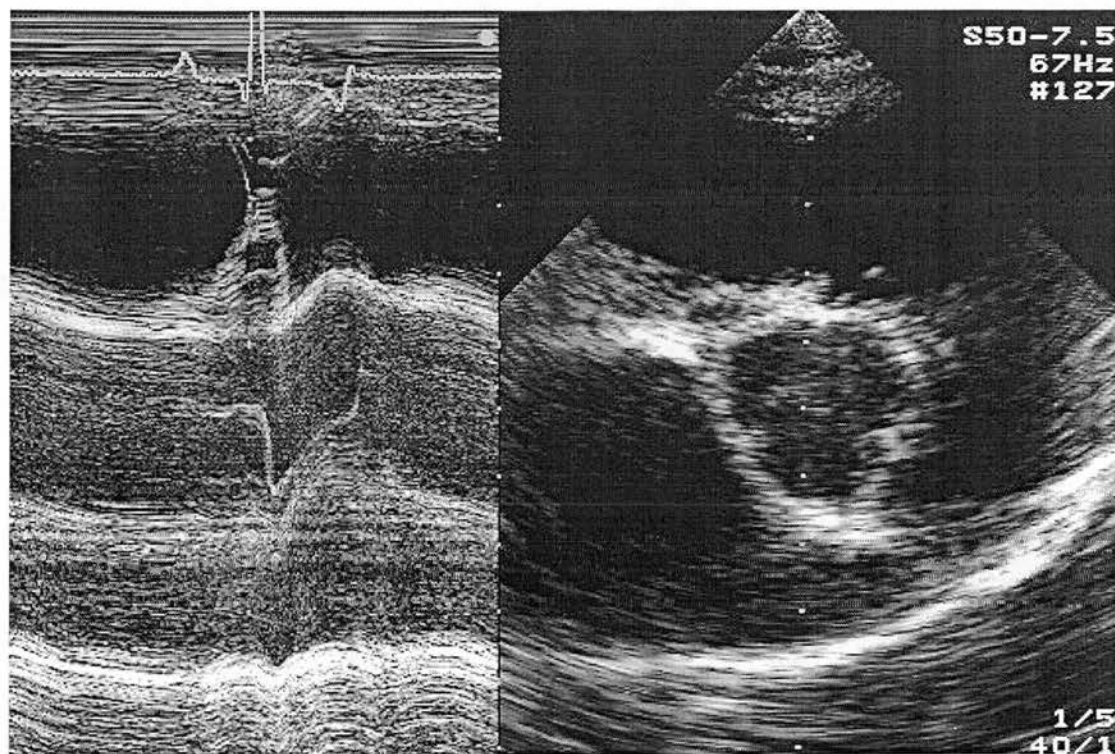


With most other machines, M-mode imaging only allows inclusion of the left auricular appendage, which may lead to underestimation of the degree of left atrial enlargement (figure 4.6).

It was interesting to note that although there was no difference in the M-mode LA:Ao ratios obtained by M-mode and 2DE short-axis images in the normal dogs, there was a significant difference in the two ratios when the values from the dogs with DCM were added, even though this was only from three dogs. It is likely that 2DE estimates of LA size are more sensitive than M-mode, as the left auricular appendage dilates relatively less than the main body of the left atrium.

There was also evidence of a difference in LA shape between the two DCM breeds, with a larger mean LA area index seen in the Dobermanns, but a wider mean LA diameter index in the cockers. This information cannot be obtained with M-mode measurements, which might lead to an underestimation of LA dilation in breeds with a relatively greater increase in apex-base dimensions.

Figure 4.6: Measurement of the left atrium (M-mode)



The m-mode cursor falls through the left auricular appendage rather than the left atrium when the aorta is bisected, thus underestimating left atrial diameter.

#### 4.6.3 Comparison of normal dogs and dogs with DCM

One of the most striking results of the 2DE values was the marked separation in EF% between the normal dogs and the dogs with DCM. This was more pronounced than with most M-mode variables used to assess ventricular function, such as FS% or EPSS. In addition, all three groups showed comparatively little intra-group variation in EF%. In view of the ubiquitous use of 2DE-derived EF% in human cardiology, this may not be a surprisingly finding, yet this variable is scarcely ever quoted in the veterinary literature.

It is interesting that some of these normal pointers had FS% values below what would be considered 'normal', yet all had 2DE EF% values that were clearly distinguishable from the DCM group. One possible explanation is that measurements of left ventricular shortening based on a single minor axis dimension do not take into account any shortening in the major axis dimension. Measurement of mitral annular motion has been suggested as a independent measurement for assessing ventricular function (Keren *et al.*, 1988) (Gulati *et al.*, 1996; Schober *et al.*, 1997).

An alternative explanation is that translational motion, which is nearly unavoidable in M-mode measurements, may lead to an underestimation of the degree of ventricular wall motion, and consequent underestimation of FS%. According to the results of a study by Douglas and others (1987) the converse is true, and M-mode is more likely to over-estimate FS%. Assuming that adequate cross-sectional planes are obtained in systole and diastole, all the available evidence suggests that 2DE

estimates of ejection fraction are more accurate than any measurements based on a single minor dimension.

#### 4.6.4 Comparison of affected Dobermanns and cocker spaniels

A list of 2DE variables found to have prognostic value are listed in table 4.14, together with the corresponding values from the dogs in this study. There was no obvious difference in systolic function between the Dobermanns and cocker spaniels, and in both groups the mean EF% was <25%. As with many of the human studies, once patients are restricted to those with severely depressed EF%, there is no further predictive prognostic power with EF%. The cocker spaniels but not the Dobermanns had a mean EDVI higher than 130 mL/m<sup>2</sup>, quoted by Diaz and others (1987) to be associated with a poorer prognosis. Both groups of affected dogs had a mean EDVI that fell within the cut-off point quoted by Juilliere and others (1988) for an improved outcome. Douglas and others (1989) reported improved survival in DCM patients with a short-to-long axis ratio of <0.76. Both Dobermanns and cockers had short-to-long axis ratios much greater than this (0.92 and 0.90, respectively), implying a greater degree of sphericity, with no difference between the two breeds. Both affected breeds had values for mean ESVI within the cut-off point quoted by Romeo and others (1989) for improved survival.

Not surprisingly, the LA areas were considerably greater in the Dobermanns in this study than in Minors' study, which only examined asymptomatic Dobermanns.

There did not appear to be significant differences between the breeds for any of the 2DE variables that could account for the difference in outcome.

Table 4.14: 2DE Prognostic Indices

Variable		↑ Survival	GSPs	Dobermanns	Cockers
EDV index	mL/ m <sup>2</sup>	<130 <sup>a</sup> <200 <sup>b</sup>	83.6 ± 11.66	129.1 ± 30.88	150.5 ± 47.38
LVDd:L		<0.76 <sup>c</sup>	0.59	0.92	0.90
ESV index	mL/ m <sup>2</sup>	<60 <sup>d*</sup> <149 <sup>e</sup>	31.9 ± 8.0	100.9 ± 26.0	120.6 ± 38.7

↑ Survival: Value associated with improved survival, GSPs: German short-haired pointers, Mean Wth: mean of free wall and septal thickness, LVDd: left ventricular diameter in diastole, PWd: left ventricular free wall in diastole, EDV index, end-diastolic volume indexed to body surface area, L: left ventricular length, ESV index, end-systolic volume indexed to body surface area, <sup>a</sup> (Diaz *et al.*, 1987), <sup>b</sup> (Juilliere *et al.*, 1988), <sup>c</sup> (Douglas *et al.*, 1989), <sup>d</sup> (Borow *et al.*, 1980), \*in human patients with mitral or aortic regurgitation, <sup>e</sup> (Romeo *et al.*, 1989)

## 4.7 CONCLUSIONS

2DE measurements of LV dimensions in dogs compare favourably with direct volume measurements and radionuclide techniques, are technically feasible and repeatable, and the software is widely available. A lack of normal reference intervals limits the current popularity of 2DE measurements, although this problem still remains an issue even with M-mode measurements because of the tremendous variation in size and conformation amongst different breeds of dogs. This study showed that 2DE measurements are easily acquired, and as reproducible as M-mode measurements.

M-mode echocardiographic measurements ignore the major axis of the LV, and fractional shortening provides a limited view of LV wall motion. In chapter 3, a notable finding was the apparently “subnormal” LV performance of the normal control group of GSPs, as demonstrated by LV fractional shortening values below

what is widely accepted as normal. The equivalent 2DE estimate of LV performance, EF%, showed no such ambiguity in this group. The current widely based reliance on M-mode derived LV fractional shortening should be reassessed, especially in view of its use as a screening tool for DCM.

2DE left atrial measurements may also offer an improvement over M-mode derived values. Problems in cursor placement in dogs result in reduced sensitivity in demonstrating left atrial enlargement, as the M-mode cursor only crosses the left auricular appendage. However, although the 2DE LA diameter measurement is highly repeatable, there will be problems in applying this measurement to anything other than serial assessment in individual animals, because of the lack of normal reference ranges. If 2DE LA measurements can be related to 2DE aortic diameters, some of these problems may be resolved.

Although a number of 2DE derived measurements have been shown to have prognostic value in human patients with DCM, the only significant differences found between Dobermanns and cocker spaniels in this study were related to body size; once these measurements were indexed to body surface area, no significant differences remained.

## CHAPTER 5: EVALUATION OF VENTRICULAR FUNCTION IN NORMAL DOGS AND DOGS WITH DCM USING DOPPLER ECHOCARDIOGRAPHY

### 5.1 INTRODUCTION

Over the last five to ten years, Doppler echocardiography has become an increasingly important tool for the non-invasive assessment of left ventricular function in human patients, animal models of cardiac disease, and in veterinary cardiology. A considerable amount of attention has been devoted to the use of Doppler echocardiography (DE) in human congestive heart failure, and dilated cardiomyopathy (DCM) in particular. Although DE has been applied to the study of systolic function, it is the area of diastolic function that has excited most interest (Oh *et al.*, 1999a).

### 5.2 AIMS

1. To measure DE variables in a group of normal German short-haired pointers, and to establish the repeatability of these measures.
2. To compare the DE results obtained in the normal dogs with those from a group of Dobermanns with DCM, and a group of English cocker spaniels with DCM.
3. To compare the results obtained in the two groups of dogs with DCM, to identify any differences in diastolic and systolic function that might merit further investigation as prognostic indicators in canine DCM.

## 5.3 BACKGROUND

### 5.3.1 Doppler-derived systolic indices in human DCM

Initial applications of DE in human DCM concentrated on systolic function. Gardin and others (1983) demonstrated reduced peak aortic flow velocity and aortic velocity time integrals in patients with DCM (Gardin *et al.*, 1983). Mean aortic acceleration and pulmonary artery velocities were also significantly depressed, but there was more overlap with values from normal subjects. Isaaz and co-workers (1989) also noted reduced aortic velocities in patients with DCM, but proposed that these velocities were augmented by additional forces relating to left ventricular dilation and increased convective acceleration forces, with consequent “ventriculoannular disproportion” effectively creating a form of relative outflow tract stenosis (Isaaz *et al.*, 1989). They therefore suggested a more sensitive measurement of systolic dysfunction would take this ventricular dilation into account, and proposed using the peak aortic velocity normalised by the ratio of the mid left ventricle to aortic cross-sectional areas (Isaaz *et al.*, 1990). Since these early reports, the number of DE studies concentrating on systolic function in human DCM has declined.

#### *5.3.1.1 Systolic time intervals*

Systolic time intervals have been used to evaluate left ventricular performance in human DCM (Ahmed *et al.*, 1972; Armstrong *et al.*, 1973), although it is only more recently that DE has been used to record them in place of phonocardiography and/or M-mode echocardiography (Burwash *et al.*, 1993). In a group of patients that included normal subjects and patients with DCM, the ratio of



pre-ejection period: left ventricular ejection time (PEP/LVET) showed a strong negative correlation with ejection fraction ( $r = -0.85$ ) (Spodick *et al.*, 1984). In a group of patients with minimal mitral regurgitation, a PEP/LVET  $>0.4$  predicted an ejection fraction  $< 55\%$  with a sensitivity of 78% and a specificity of 92% (Burwash *et al.*, 1993).

Although systolic dysfunction is clearly important in human DCM, calculation of ejection fraction by two-dimensional echocardiography (or even M-mode echocardiography) is still the preferred means of non-invasive assessment. In contrast, DE measurements of diastolic filling have been the subject of intense focus in human DCM.

### 5.3.2 Doppler-derived diastolic indices in human DCM

The close association between left ventricular filling pressures and functional class of heart failure has led to close scrutiny of the clinical significance of diastolic filling parameters in DCM (Vanoverschelde *et al.*, 1990). A number of early studies showed the same findings: patients with less severe symptoms of heart failure or with lower filling pressures had transmitral filling shifted towards the end of diastole, whereas patients with severe heart failure or elevated filling pressures had enhanced early filling with a minimal atrial contribution to filling. Moderately affected patients often appeared to have normal filling patterns (Lavine and Arends, 1989; St Goar *et al.*, 1991; Vanoverschelde *et al.*, 1990).

The reasons for the “normalisation” of filling patterns with increasing severity of heart failure were initially poorly understood, and a number of explanations were proposed. Many of the most symptomatic patients had substantial

mitral regurgitation, and a correlation was found in a number of studies between severity of mitral regurgitation, elevated left atrial pressures, and an increased ratio of mitral early (E) velocity to atrial (A) velocity (Lavine and Arends, 1989; Takenaka *et al.*, 1986b; Vanoverschelde *et al.*, 1990). It seemed logical that severe mitral regurgitation would increase left atrial pressures compared to patients without mitral regurgitation, leading to an increased left atrial-left ventricular pressure gradient at the start of diastole and higher E wave velocities. However, this theory did not explain why there were some DCM patients in the study by Lavine and Arends (1989) with normal filling pressures and normal E/A ratios, when most asymptomatic DCM patients had a reduced E/A ratio (Lavine and Arends, 1989).

In a study by David and co-workers (1989), transmitral Doppler patterns were measured simultaneously with direct micromanometer measurements of left atrial and left ventricular pressures in seven DCM patients (David *et al.*, 1989). An infusion of amrinone was then given to alter left ventricular contractility and relaxation. Left ventricular fractional shortening did not change, but velocity of circumferential fibre shortening increased, and relaxation improved, as shown by a reduction in the time constant of isovolumic relaxation,  $\tau$ . The mean E/A ratio decreased with amrinone, and the authors interpreted this as indicating “a drug-induced decrease in LV compliance”. At this time, many still naively regarded a reduced mitral E/A ratio as evidence of “abnormal diastolic function”, without making a clear distinction between abnormal relaxation and abnormal compliance. In fact, in David’s study there was a profound fall in left atrial pressure with amrinone infusion, as well as a decrease in end-diastolic pressure. This resulted in a decreased early diastolic transmitral pressure gradient (reducing the E wave

velocity), and an increased gradient in late diastole associated with a shift to a more compliant portion of the diastolic pressure-volume relationship (increasing the A wave velocity).

Appleton and Hatle's work (1992) did much to explain the reasons for the range of transmitral filling patterns observed in human patients with ventricular dysfunction (Appleton and Hatle, 1992). Patients were classified into three groups:

- (1) *abnormal relaxation*, with a reduced mitral E/A ratio, prolonged mitral deceleration time and isovolumic relaxation time, normal filling pressures, and minimal symptoms.
- (2) "*pseudonormal*", with a normal mitral E/A ratio, normal deceleration time and isovolumic relaxation time, and mildly elevated filling pressures or mildly abnormal chamber compliance.
- (3) "*restrictive*", with an increased E/A ratio, short deceleration time and isovolumic relaxation time, and increased left atrial pressures and left ventricular chamber stiffness.

Abnormal relaxation was believed to be present in all three groups, but was masked in the pseudonormal and restrictive groups by increased filling pressures and decreased chamber compliance.

Thomas and Weyman (1991) developed a mathematical model of left ventricular filling, using a lumped parameter model to predict the effects of changes in atrial pressure, ventricular relaxation, and intrinsic myocardial stiffness, amongst other factors (Thomas and Weyman, 1991). These studies provided further evidence for Appleton and Hatle's interpretations of transmitral Doppler flow. Direct

demonstration of the progression of diastolic dysfunction was subsequently shown in a canine model of myocardial failure (Ohno *et al.*, 1994). Myocardial failure was induced by rapid ventricular pacing, and left atrial and left ventricular pressures were measured directly. Early in the course of failure, peak early filling decreased from baseline values as left ventricular relaxation deteriorated. Subsequently peak early filling returned to normal values, and then increased in association with increased left atrial pressures and increased left ventricular chamber stiffness, despite persistently abnormal left ventricular relaxation.

Numerous other studies have confirmed that these patterns of transmitral flow are directly applicable to patients with DCM (Lapu-Bula *et al.*, 1998; Rihal *et al.*, 1994; Shen *et al.*, 1992; Vanoverschelde *et al.*, 1990; Werner *et al.*, 1996; Werner *et al.*, 1993; Xie *et al.*, 1994).

### 5.3.3 Doppler echocardiographic prognostic indicators

#### *5.3.3.1 Diastolic variables and prognosis*

One of the main reasons for the interest in transmitral Doppler filling patterns in DCM patients is the link with prognosis. Patients with elevated pulmonary capillary wedge (left atrial) pressures or elevated left ventricular end-diastolic pressures have consistently been shown to have worse survival rates (Diaz *et al.*, 1987; Juilliere *et al.*, 1988; Keogh *et al.*, 1990; Ogasawara *et al.*, 1987; Stevenson *et al.*, 1990; Unverferth *et al.*, 1984). There is a close association between increased filling pressures and a restrictive pattern of transmitral filling in patients with DCM. Correlation coefficients of pulmonary capillary wedge pressure to mitral E/A ratio have ranged from 0.69 (Pozzoli *et al.*, 1995) to 0.94

(Vanoverschelde *et al.*, 1990), lending support to the use of DE as a tool for investigating prognostic risk (Lavine and Arends, 1989). In numerous studies of patients with DCM, Cox proportional-hazards analysis has shown Doppler transmitral filling patterns (and mitral deceleration time in particular) to be amongst the strongest predictors of outcome (Giannuzzi *et al.*, 1996; Lapu-Bula *et al.*, 1998; Pinamonti *et al.*, 1993; Rihal *et al.*, 1994; Shen *et al.*, 1992; Werner *et al.*, 1996; Werner *et al.*, 1994; Xie *et al.*, 1994). In all these studies, a high mitral E/A ratio, and a short mitral deceleration time (i.e. a restrictive filling pattern) was predictive of a worse outcome.

#### *5.3.3.2 Other Doppler-derived prognostic indicators*

Additional Doppler-derived variables that have been associated with a poor outcome in human DCM include peak velocity of tricuspid regurgitation, which can be used to calculate pulmonary artery systolic pressure (Abramson *et al.*, 1992), and the severity of mitral regurgitation (Blondheim *et al.*, 1991; Junker *et al.*, 1993). For the most part, DE systolic indices have not proved useful in prognosis, although Keren and others (1992) measured stroke volume using DE in a group of patients with DCM randomised to receive either captopril or placebo (Keren *et al.*, 1992). Stroke volume increased only in the captopril-treated group, but the most important findings in this study were the changes in diastolic filling patterns.

### 5.3.4 Assessment of canine DCM with Doppler echocardiography

#### 5.3.4.1 *Systolic function*

Doppler echocardiography is a relatively new technique in veterinary cardiology, and there are very few published reports of its application to canine DCM. One study showed that when dogs with DCM were compared to normal dogs, they had significantly lower peak aortic and pulmonary artery velocities, and rates of aortic and pulmonary artery acceleration (Darke *et al.*, 1993). Systolic time intervals have also been reported for dogs with DCM using DE; although reported values for PEP/LVET are variable, ranging from 0.25 (Amberger and Lombard, 1998) to 0.85 (Dukes McEwan, 1998a), although there may have been technical errors associated with the latter result (Amberger and Lombard, 1998).

#### 5.3.4.2 *Diastolic function*

In one report comparing dogs with overt DCM and normal dogs, the affected dogs had a higher mitral E/A ratio and shorter E wave duration (Darke *et al.*, 1993). Although there are relatively few reports of Doppler filling patterns in spontaneous canine DCM, they have been evaluated in dogs considered to have early (or occult) DCM (Dukes McEwan, 1998b; Minors and O'Grady, 1998). Minors and O'Grady (1998) did not find any differences in resting mitral E/A ratios between normal Dobermanns and Dobermanns considered to have occult DCM, although the isovolumic relaxation time was prolonged in dogs that went on to develop occult DCM. Dukes McEwan (1998) found a significant increase in mitral E/A ratio in Newfoundlands with DCM (although this group included overt and occult DCM) (Dukes McEwan, 1998b).

#### *5.3.4.3 Doppler echocardiographic prognostic indicators in canine DCM*

Borgarelli and others (1997). attempted to relate echocardiographic variables to prognosis in a series of 31 dogs with DCM (Borgarelli *et al.*, 1997). Dogs were classified as having a restrictive or nonrestrictive Doppler transmitral filling pattern, but although survival was greater in the nonrestrictive (delayed relaxation) group, the difference was not significant. The most reliable predictor of survival in this study was velocity of mitral regurgitation: dogs with values  $> 4$  m/s had a one year survival rate of 83%, compared with 23% survival in dogs with mitral regurgitation velocities  $< 4$  m/s ( $p < 0.001$ ) (Borgarelli *et al.*, 1997).

#### *5.3.4.4 Doppler echocardiography in canine models of DCM*

The study by Ohno and co-workers (1994) in dogs with myocardial failure induced by rapid ventricular pacing did not actually assess Doppler-derived transmitral filling variables, although diastolic function was assessed invasively (Ohno *et al.*, 1994). Transmitral flow patterns have been studied in dogs where systolic failure was induced by a stepwise injection of microspheres into the left coronary artery (Yamamoto *et al.*, 1993b). As with Ohno's study, with the onset of mild systolic dysfunction, there was a concurrent increase in the time constant of isovolumic relaxation ( $\tau$ ). In addition there was an increase in mean left atrial pressure, and an increase in minimum left ventricular pressure, such that the mitral E/A ratio was unchanged. With more severe left ventricular dysfunction, mean left atrial pressure and minimum left ventricular pressure increased further, and there was

a non-significant increase in mitral E/A ratio. Despite the lack of change in E/A ratios, the mitral deceleration rate showed a significant stepwise increase, and the mitral deceleration time became progressively shorter with increasing left ventricular dysfunction. There is another report of mitral E/A ratios in dogs treated with doxorubicin, but the numbers of dogs were small (three dogs in one treatment group), and E/A ratios decreased in two dogs and increased in the third (Hanai *et al.*, 1996). The study was poorly designed, and one should probably not attempt to draw any conclusions from the results.

## 5.4 MATERIALS AND METHODS

### 5.4.1 Equipment

All Doppler echocardiographic studies were carried out using a Vingmed CFM 700 ultrasound system (Diasonics, Sonotron, Bedford, UK) using a 2.25 MHz annular array transducer that operated at a frequency of 2.0 MHz for Doppler. A simultaneous ECG was recorded in all studies (limb lead II) using pre-gelled adhesive electrodes attached to the main pads of the feet, or to the limbs above the carpus and tarsus (F60, Skintact ECG pads, HA West Ltd, 41 Watson Crescent, Edinburgh, UK ). Electrodes were further secured using permeable non-woven synthetic adhesive tape (Micropore, 3M Health Care Ltd, 3M House, Morley St, Loughborough, Leics, UK). Studies were recorded onto VHS videotape by a Panasonic AG 6200 video cassette recorder. Still transparencies could be recorded onto 35 mm film using a freeze frame recorder (Polaroid UK Limited, Ashley Road, St Albans, Herts, UK).



#### *5.4.1.1 Echocardiograph settings*

Spectral Doppler recordings could be made using continuous wave Doppler or using high pulse repetition frequency (HPRF). When in the HPRF mode, blood flow velocities greater than 1.5 m/s could be displayed without aliasing, as additional sample volumes would be automatically introduced as the velocity scale was increased. Although this introduced range ambiguity, colour flow mapping usually confirmed the site of maximal velocity flow when blood flow exceeded 1.5 m/s. For recording blood flow velocities over 4.0 m/s, continuous wave DE was generally used. For HPRF Doppler, the sample volume size was set at 2.5 mm.

The spectral Doppler reject levels were generally set at minimum, and gain levels were set at a level where faint signals were just present in the background. A low velocity filter was used to minimise low velocity signals near the baseline; this was kept at a minimum when recording normal laminar flow, but was increased for high velocity flow. The compression level (which adjusted the relationship between signal amplitude and the grey scale level intensity) was pre-set, and kept constant for each study. The baseline could also be moved, to allow maximum display of the spectral signal. The sample volume was aligned using the 2D image, which was then frozen once spectral signal acquisition began. Further adjustments were then made to probe alignment using the audio signal and visual appearance of the spectral envelopes, until minimal spectral dispersion was displayed.

The colour flow mapping displayed information about blood flow velocity and direction by superimposing Doppler information on a grey-scale 2D image. Blood flow moving towards the transducer was coded as red, and blood flow away from the transducer was coded as blue. Aliasing occurred when the Nyquist limit

was exceeded. Blood flow was coded as green when a wide signal bandwidth or highly aliased signal was present, indicating disturbed flow. During colour flow mapping, the colour flow sector was kept at  $35^\circ$  to maintain maximum frame rate. The frame rate varied between 13 and 16 frames/sec. At the standard depths used, the Nyquist limit could reach as high as 1.3 m/s with the 2.25 MHz probe. The reject value was set at 0.24 m/s, and the gain was set at the maximal level that did not produce extraneous artefacts.

#### 5.4.2 Methods

Dogs were scanned unsedated, and manually restrained in right lateral recumbency on a purpose-built table. The table consisted of a metal base with a removable rigid Perspex top, with had a V-shaped section removed halfway along the edge of one of the long sides. This was to facilitate access for the transducer to the dependent part of the thorax, so that right parasternal views were obtained with the dog lying in right lateral recumbency.

A series of views was obtained in each dog in strict sequence, starting with the dog in right lateral recumbency. A right parasternal long axis view were recorded first, and colour flow mapping was used to identify any mitral or tricuspid regurgitation. A short axis view was obtained of the heart base, and colour flow mapping was used to determine the optimum image for recording pulmonary artery flow by spectral Doppler. Recordings were then made of aortic flow from the subcostal window (when technically feasible).

The dog was then moved into left lateral recumbency, and 4-chamber and 2-chamber views were recorded from the left apical (caudal parasternal) window. Colour flow mapping was used to identify the optimum plane for transmitral flow, and the presence of any mitral regurgitation visible from these views was noted. Spectral Doppler recordings were made of transmitral flow from an apical 4-chamber view, and continuous wave Doppler was used to record any mitral regurgitation present. A similar process was repeated for the tricuspid valve at both an apical 4-chamber view, and from a more cranial intercostal space. Spectral recordings were made of aortic flow from a left apical (caudal parasternal) three-chamber view. From a left cranial parasternal view, alignment was obtained with the pulmonary artery and spectral recordings were made.

#### 5.4.3 Protocol

##### *5.4.3.1 Normal GSH pointers, reproducibility studies*

All dogs were clinically normal on physical examination, and had no history that would suggest cardiac disease. The full details of these dogs are described in chapter 2. Eleven dogs were scanned on one occasion only, and two dogs were scanned on each of five successive days. Subcostal recordings of aortic velocity were obtained in 10/13 dogs.

##### *5.4.3.2 Dogs with DCM*

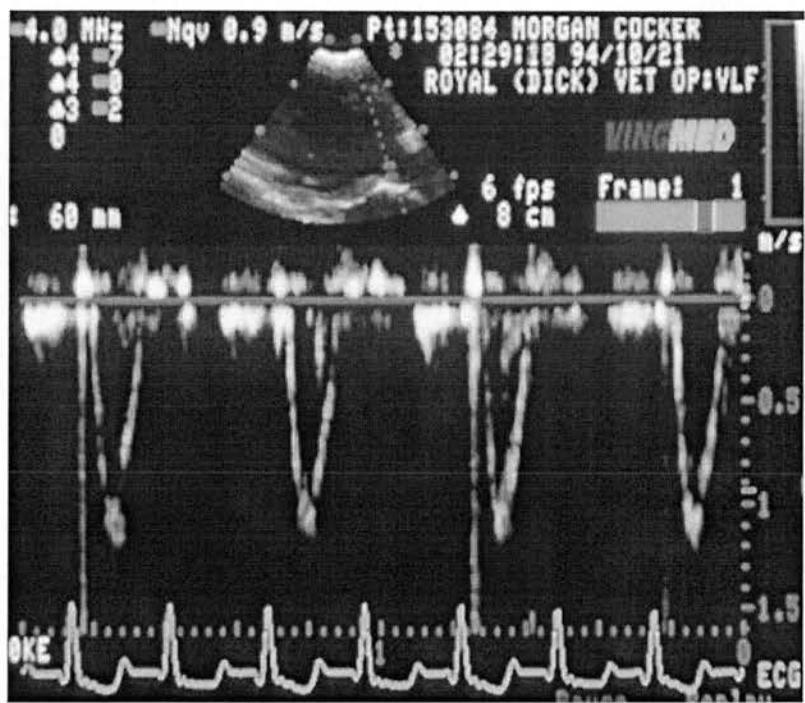
The details of the dogs with DCM are presented in chapter 2: they comprised 11 Dobermanns and 9 cocker spaniels. Subcostal recordings of aortic

flow proved difficult to record (especially in the Dobermanns) and it was decided not to attempt to record from this view.

#### 5.4.4 Measurements of Doppler echocardiographic variables

Where applicable, measurements were made in accordance with the Canadian Consensus recommendations (Rakowski *et al.*, 1996) unless otherwise indicated. Measurements were made off-line from the recorded video cassette tapes. The Vingmed system integral measuring software could be employed by entering a calibration code recorded in the lower left corner of the recorded image when prompted. For each variable, a minimum of five beats was averaged (ten beats in the presence of sinus arrhythmia or atrial fibrillation). Ectopic beats or post-extrasystolic beats were excluded, as were periods of pulsus alternans (figure 5.1).

Figure 5.1: Spectral Doppler echocardiogram of aortic blood flow in a cocker spaniel with dilated cardiomyopathy.



Aortic velocity recordings in cocker 2, showing severe pulsus alternans. Aortic blood flow is only evident in alternate beats, despite a normal sinus rhythm.

Spectral Doppler measurements were made with electronic callipers guided by the tracker ball. Systolic time intervals were recorded from aortic blood flow recordings. Pre-ejection period (PEP) was measured from the onset of the Q wave to the onset of aortic flow. Ejection time (ET) was measured from the start of aortic flow to the end of the aortic systolic signal. Mean aortic acceleration was measured from the onset of aortic flow to peak velocity. No attempt was made to measure maximum aortic acceleration. The aortic spectral envelopes were traced along the modal velocity (assumed to be the brightest area of the outline of the envelope) to yield time velocity integral (VTI). Measurements were also made of pulmonary artery velocities, mean acceleration and ejection time, and of mitral and tricuspid inflow velocities. For transmitral flow, measurements were made of E wave and A wave velocities, E wave deceleration slope, and E wave deceleration time. When there was no period of diastasis, and the mitral E and A waves abutted each other, the deceleration time was taken as the interval from the peak of the E wave to a point where the deceleration slope would have met the baseline on extrapolation.

Colour flow was used to assess atrioventricular valvular regurgitation, and a score was assigned according to spatial mapping (Rivera *et al.*, 1994). A score of 1+ was assigned when the largest jet in any plane filled less than 20% of the atrium; 2+ denoted a jet that filled between 20-40% of the receiving chamber; and 3+ jets filled over 40% of the receiving chamber (Helmcke *et al.*, 1987).

#### 5.4.4.1 Calculation of derived variables

Measurements of isovolumic relaxation time were made indirectly, by one of two ways:

Formula 5.1

$$\text{IVRT} = [\text{Q} - \text{MV inflow}] - [\text{Q} - \text{AoV closure}]$$

Formula 5.2

$$\text{IVRT} = [\text{Q} - \text{MV inflow}] - [\text{PEP} + \text{ET}]$$

where Q – MV inflow is the interval from the Q wave on the ECG to the onset of mitral inflow, Q – AoV closure is the interval from the Q wave on the ECG to the end of aortic flow, PEP is pre-ejection period, and ET is ejection time. This differs from the Canadian Consensus recommendations (Rakowski *et al.*, 1996).

Left ventricular rate of pressure rise was calculated from spectral recordings of mitral regurgitation (Chung *et al.*, 1992). The time (dt) taken for the mitral regurgitant velocity (in seconds) to accelerate from 1m/s to 3 m/s is the same as the time taken for the left ventricle to generate a pressure gradient of  $4 \times (3^2 - 1^2) = 32$  mmHg (from the modified Bernoulli equation). Thus the rate of change in pressure can be calculated for this period in mmHg/sec by the following formula:

Formula 5.3

$$dP/dt = 32 / dt$$

#### 5.4.5 Statistical methods

All values are expressed as mean  $\pm$  SD. Coefficients of variation were calculated for the repeated measures in the two normal GSH pointers by dividing the standard deviation by the mean, and multiplying by 100 to express the result as a percentage. Coefficients of variation less than 10% were considered good. The values were compared between GSH pointers, Dobermanns and cocker spaniels by one way analysis of variance when data were normally distributed with equal variance, and by Kruskal-Wallis one way analysis of variance when a test for normality or equal variances failed. When a significant difference was found between groups, multiple pair-wise comparisons were made using a Tukey test for normally distributed data, or Dunn's method following a Kruskal-Wallis test. The significance level was set at  $p < 0.05$ . All statistical analyses were carried out using a proprietary statistical software programme (SigmaStat 2.0, Jandel Scientific, San Rafael, CA, USA). Graphs were plotted using SigmaPlot (Jandel Scientific, San Rafael, CA, USA).

### 5.5 RESULTS

#### 5.5.1 German short-haired pointers

Aortic velocity variables from the left apical view for the normal GSH pointers are presented in table 5.1, pulmonary artery variables in table 5.2, and mitral and tricuspid valve values are presented in table 5.3. Aortic velocities from the subcostal window are listed in Appendix 3. Aortic velocities were significantly higher ( $p < 0.001$ ) from the subcostal window ( $1.4 \pm 0.23$  m/s versus  $1.24 \pm 0.19$  m/s) when compared by a paired t-test. The subcostal velocity time integrals and



rates of acceleration ( $dV/dt$ ) were also higher, and the acceleration times shorter. However, there was no significant difference in PEP/LVET between the two views (subcostal  $0.32 \pm 0.05$ , left apex  $0.30 \pm 0.04$ ). Pulmonary artery (PA) velocity and acceleration were similar whether recorded from the right or left parasternal views (PA velocity from right  $0.90 \pm 0.17$  m/s, from left  $0.86 \pm 0.16$  m/s; acceleration from right  $12.3 \pm 3.2$  ms<sup>-2</sup>, from left  $12.4 \pm 3.3$  ms<sup>-2</sup>). The isovolumic relaxation time (IVRT) was measured using both subcostal and left apical aortic recordings in 10/13 GSH pointers. In this group of 10 dogs, the IVRT was significantly different ( $p < 0.001$ ) when calculated from the subcostal recording ( $0.056 \pm 0.018$  sec, versus  $0.041 \pm 0.02$  sec). The mean value for IVRT from the apical view for all 13 GSH pointers was  $0.036 \pm 0.021$  sec.

Table 5.1: DE measurements of left apical aortic flow in individual GSH pointers.

Dog	Ao vel ms <sup>-1</sup>	VTI cm	HR /min	dV/dt ms <sup>-2</sup>	acc dt s	ET s	PEP s	PEP/ET	SC IVRT s	LAp IVRT s
1	1.08	12.54	69.7	20.86	0.055	0.207	0.066	0.319	0.037	0.023
2	1.17	13.66	54.9	27.6	0.046	0.208	0.061	0.294	0.040	0.028
3	1.59	*19.55	66.9	36.09	0.048	0.208	0.065	0.313	0.049	0.025
4	1.03	10.18	80.6	19.08	0.058	0.191	0.059	0.309	0.067	0.043
5	1.43	13.85	89.7	27.65	0.054	0.188	0.051	0.271	0.056	0.029
6	1.32	13.94	109.8	33.04	0.044	0.179	0.069	0.385	NR	0.044
7	0.98	8.99	98.9	17.22	0.06	0.192	0.054	0.281	NR	0.012
8	1.15	10.49	104.1	34.6	0.038	0.176	0.061	0.347	0.061	0.063
9	1.14	11.79	95.4	27.97	0.044	0.208	0.050	0.240	0.096	0.086
10	1.35	15.52	67.9	29.67	0.0514	0.206	0.059	0.286	NR	0.018
11	1.1	11.37	79.4	22.93	0.05	0.197	0.064	0.325	0.047	0.031
12	1.5	15.93	86.3	33.11	0.049	0.198	0.054	0.273	0.038	0.034
13	1.29	15.49	83.3	24.89	0.054	0.211	0.059	0.280	0.067	0.045
mean	1.24	13.33	83.61	27.29	0.05	0.198	0.059	0.302	0.056	0.036
SD	0.19	2.88	16.06	6.05	0.01	0.012	0.006	0.037	0.018	0.021

\*Indicates value >2SD from the mean. DE: Doppler echocardiography, GSH: German Short-Haired, Ao vel: aortic velocity, VTI: velocity time integral, HR: heart rate, dV/dt: aortic flow acceleration, acc dt: aortic flow acceleration time, ET: left ventricular ejection time, PEP: left ventricular pre-ejection period, SC IVRT: left ventricular isovolumic relaxation time from subcostal window, LAp IVRT: left ventricular isovolumic relaxation time from left apex, SD: standard deviation, NR: not recorded.

Table 5.2: DE pulmonary artery measurements in individual GSH pointers.

Dog	PA from R. parasternal			PA from L. parasternal		
	vel ms <sup>-1</sup>	dV/dt ms <sup>-2</sup>	ET s	vel ms <sup>-1</sup>	dV/dt ms <sup>-2</sup>	ET s
1	0.65	6.62	0.218	0.59	7.31	0.244
2	0.73	11.39	0.247	0.81	9.87	0.256
3	1.08	14.42	0.237	1.08	14.64	0.23
4	0.96	13.85	0.23	0.68	10.4	0.22
5	1.15	19.39	0.225	NR	NR	NR
6	0.79	11.53	0.21	NR	NR	NR
7	1	12.99	0.19	0.99	16.08	0.21
8	0.87	12.15	0.225	0.77	13.85	0.208
9	0.84	8.55	0.222	1.02	18	0.226
10	0.74	NR	NR	0.93	13.99	0.227
11	0.81	10.69	0.224	0.73	9.54	0.218
12	0.87	11.99	0.216	0.84	9.51	0.235
13	1.22	14.39	0.23	1.05	13.55	0.239
mean	0.90	12.33	0.223	0.86	12.43	0.228
SD	0.17	3.20	0.014	0.16	3.30	0.015

DE: Doppler echocardiography, GSH: German short-haired, PA: pulmonary artery, R. parasternal: right parasternal view, L. parasternal: left parasternal view, vel: velocity, dV/dt: acceleration, ET: ejection time, SD: standard deviation, NR: not recorded.

Table 5.3: Mitral and tricuspid inflow DE measurements in individual GSH pointers.

Dog	Mitral						Tricuspid		
	E vel ms <sup>-1</sup>	A vel ms <sup>-1</sup>	E/A	E DT s	E dur s	E decel ms <sup>-2</sup>	E vel ms <sup>-1</sup>	A vel ms <sup>-1</sup>	E/A
1	0.68	0.42	1.62	0.068	0.149	10.72	0.49	0.26	1.88
2	0.61	0.43	1.42	0.090	0.184	7.46	0.52	0.27	1.93
3	0.49	0.43	1.14	0.072	0.153	7.12	0.55	0.33	1.67
4	0.58	0.36	1.61	0.096	0.191	6.99	0.47	0.27	1.74
5	0.90	0.53	1.10	0.081	0.173	12.49	0.75	0.50	1.50
6	0.69	0.43	1.60	0.074	0.156	10.37	0.43	0.36	1.19
7	0.87	0.42	2.07	0.082	0.150	11.41	0.61	0.24	2.54
8	0.62	0.46	1.35	0.108	0.178	5.89	0.50	0.32	1.56
9	0.62	0.42	1.48	0.085	0.144	7.60	0.47	0.48	0.98
10	0.77	0.51	1.51	0.090	0.163	7.87	0.77	0.51	1.51
11	0.60	0.31	1.94	0.098	0.192	6.96	0.60	0.25	2.40
12	0.84	0.45	1.87	0.091	0.159	9.60	0.78	0.41	1.90
13	0.68	0.47	1.45	0.077	0.165	10.52	0.79	0.43	1.84
mean	0.69	0.43	1.55	0.09	0.17	8.85	0.59	0.36	1.74
SD	0.12	0.06	0.29	0.01	0.02	2.09	0.13	0.10	0.43

DE: Doppler echocardiography, GSH: German short-haired, MV: mitral valve, TV: tricuspid valve, E vel: velocity of early filling, A vel: velocity of atrial filling, E/A: ratio of early to atrial filling velocities, E DT: E wave deceleration time, E dur: duration of mitral E wave, E decel: mitral E wave deceleration, SD: standard deviation.

#### 5.5.1.1 Repeatability studies

The repeated measurements in two normal dogs are shown in tables 5.4 to 5.7. Coefficients of variation was less than 10% in at least one dog for all the subcostal aortic measurements except acceleration, for all the left apical aortic measurements (except acceleration, acceleration time, and pre-ejection period), PA ejection time (both views) and PA velocity from the left, tricuspid E velocity, mitral E velocity, and mitral E duration. Coefficients of variation exceeded 15% for the

following variables: pulmonary artery acceleration (one dog), subcostal aortic acceleration (one dog), subcostal aortic acceleration time (one dog), isovolumic relaxation time (both views, both dogs), mitral A wave velocity (one dog), mitral E/A ratio (one dog), mitral E deceleration time (both dogs), mitral E deceleration (both dogs) and tricuspid A velocity (one dog). The repeatability of the DE variables overall was lower than with M-mode and two-dimensional echocardiography (2DE) when assessed by comparing the coefficients of variation using a Kruskal-Wallis test on ranks ( $p = 0.002$ ), although the median DE coefficient of variation differed significantly from only the 2DE median value in pairwise comparisons (M-mode 6.8%, 2DE 6.0%, DE 10.3%).

Table 5.4: Repeated DE left apical aortic flow values in normal GSH pointers.

	Ao vel ms <sup>-1</sup>		VTI cm		dV/dt ms <sup>-2</sup>		acc dt s		ET s		PEP s		PEP/ET	
	Dog 1	Dog 2	Dog 1	Dog 2	Dog 1	Dog 2	Dog 1	Dog 1	Dog 2	Dog 2	Dog 1	Dog 2	Dog 1	Dog 2
Day 1	1.35	1.29	14.26	15.49	23.41	24.89	0.060	0.054	0.196	0.211	0.042	0.059	0.21	0.28
Day 2	1.50	1.14	15.93	12.91	33.11	20.70	0.049	0.058	0.198	0.205	0.054	0.068	0.27	0.33
Day 3	1.26	1.06	14.07	12.92	22.99	21.77	0.058	0.051	0.202	0.215	0.058	0.064	0.29	0.30
Day 4	1.32	1.18	15.15	13.63	21.71	23.23	0.064	0.053	0.201	0.212	0.047	0.061	0.23	0.29
Day 5	1.28	1.31	15.15	15.23	25.27	23.15	0.053	0.059	0.202	0.213	0.056	0.055	0.26	0.26
mean	1.34	1.19	14.91	13.44	25.30	23.72	0.057	0.053	0.200	0.211	0.051	0.060	0.25	0.28
SD	0.09	0.09	0.68	1.84	4.17	2.78	0.006	0.006	0.003	0.003	0.006	0.006	0.03	0.03
CV%	6.4	8.0	4.6	13.7	16.5	11.7	10.5	11.6	1.4	1.6	11.8	10.3	10.5	11.2

DE: Doppler echocardiography, GSH: German short-haired, Ao vel: aortic velocity, VTI: velocity time integral, dV/dt: aortic flow acceleration, acc dt: aortic flow acceleration time, ET: left ventricular ejection time, PEP: left ventricular pre-ejection period, SD: standard deviation, CV%: coefficient of variation.

Table 5.5: Repeated DE values for pulmonary artery in normal GSH pointers.

	PA from R. parasternal view						PA from L. parasternal view					
	vel		dV/dt		ET		vel		dV/dt		ET	
	ms <sup>-1</sup>		ms <sup>-2</sup>		s		ms <sup>-1</sup>		ms <sup>-2</sup>		s	
	Dog 1	Dog 2	Dog 1	Dog 2	Dog 1	Dog 2	Dog 1	Dog 2	Dog 1	Dog 2	Dog 1	Dog 2
Day 1	1.14	1.22	13.04	14.39	0.221	0.230	NR	1.05	NR	13.55	NR	0.239
Day 2	0.87	0.97	11.99	10.80	0.216	0.242	0.84	1.17	9.51	16.12	0.235	0.230
Day 3	0.85	0.91	9.63	10.16	0.248	0.252	0.82	0.86	11.28	13.15	0.237	0.222
Day 4	0.93	1.06	9.85	11.68	0.236	0.230	0.89	NR	12.72	NR	0.227	NR
Day 5	0.77	0.95	9.08	10.86	0.226	0.240	0.93	0.92	11.10	11.70	0.240	0.230
mean	0.91	1.02	10.72	11.58	0.229	0.239	0.87	1.00	11.15	13.63	0.235	0.230
SD	0.14	0.12	2.01	1.66	0.013	0.009	0.05	0.14	1.31	1.84	0.006	0.007
CV%	15.03	12.09	18.74	14.36	5.596	3.865	5.71	13.83	11.78	13.50	2.369	3.017

DE: Doppler echocardiography, GSH: German short-haired, PA: pulmonary artery, R. parasternal: right parasternal, L. parasternal: left parasternal, vel: velocity, dV/dt: acceleration, ET: ejection time, SD: standard deviation, NR: not recorded, CV%: coefficient of variation.

Table 5.6: Repeated DE values for heart rate, tricuspid valve and IVRT in normal GSH pointers.

	HR				Tricuspid				IVRT			
	/min		E vel		A vel		E/A		Subcostal view		L. Apical view s	
	ms <sup>-1</sup>		ms <sup>-1</sup>		ms <sup>-1</sup>				s		s	
	Dog 1	Dog 2	Dog 1	Dog 2	Dog 1	Dog 2	Dog 1	Dog 2	Dog 1	Dog 2	Dog 1	Dog 2
Day 1	87.4	82.2	0.76	0.79	0.42	0.43	1.81	1.84	0.026	0.067	0.28	0.45
Day 2	78.4	82.5	0.78	0.72	0.41	0.29	1.90	2.48	0.038	0.035	0.34	0.44
Day 3	78.7	73.2	0.73	0.77	0.40	0.31	1.83	2.52	0.038	0.066	0.29	0.53
Day 4	75.4	63.3	0.73	0.72	0.36	0.32	2.03	2.25	0.026	0.048	0.33	0.43
Day 5	62.8	77.0	0.72	0.74	0.30	0.36	2.40	2.05	0.035	0.057	0.35	0.65
mean	76.5	76.5	0.74	0.75	0.38	0.33	1.99	2.30	0.033	0.055	0.32	0.49
SD	9.6	7.5	0.04	0.03	0.04	0.06	0.25	0.31	0.006	0.013	0.07	0.09
CV%	12.6	9.6	5.60	3.76	11.80	16.78	12.56	13.39	18.9	24.5	20.0	18.0

DE: Doppler echocardiography, IVRT: left ventricular isovolumic relaxation time, GSH: German short-haired, HR: heart rate, L.: left, E vel: velocity of early filling, A vel: velocity of atrial filling, E/A: ratio of early to atrial filling velocities, SD: standard deviation, CV%: coefficient of variation.

Table 5.7: Repeated DE values for mitral valve inflow in GSH pointers

	E vel $\text{m s}^{-1}$		A vel $\text{m s}^{-1}$		E/A		E DT s		E dur s		E decel s	
	Dog 1	Dog 2	Dog 1	Dog 2	Dog 1	Dog 2	Dog 1	Dog 2	Dog 1	Dog 2	Dog 1	Dog 2
Day 1	0.86	0.68	0.50	0.47	1.72	1.45	0.057	0.077	0.13	0.17	16.23	10.52
Day 2	0.84	0.61	0.45	0.45	1.87	1.36	0.091	0.102	0.16	0.19	9.60	6.17
Day 3	0.69	0.60	0.36	0.28	1.92	2.14	0.069	0.079	0.13	0.16	16.51	7.96
Day 4	0.88	0.70	0.43	0.43	2.05	1.63	0.067	0.083	0.14	0.17	13.91	9.22
Day 5	0.77	0.62	0.34	0.36	2.26	1.72	0.082	0.091	0.16	0.17	9.66	7.86
mean	0.81	0.65	0.42	0.40	1.96	1.65	0.073	0.082	0.14	0.16	13.18	8.35
SD	0.08	0.04	0.06	0.07	0.19	0.27	0.013	0.015	0.02	0.02	3.15	1.63
CV%	10.05	6.34	14.34	17.46	9.52	16.49	17.81	18.09	11.64	9.17	23.93	19.51

DE: Doppler echocardiography, GSH: German short-haired, E vel: early mitral filling velocity, A vel: mitral atrial filling velocity, E/A: ratio of early to atrial filling velocities, E DT: mitral E wave deceleration time, E dur: duration of mitral E wave, E decel: mitral E wave deceleration, SD: standard deviation, CV%: coefficient of variation.

5.5.2 Dobermanns with DCM

Aortic flow measurements for Dobermanns with DCM are displayed in table 5.8, mitral inflow variables are presented in table 5.9, and right heart variables and atrioventricular valve regurgitation measurements are listed in table 5.10.

Table 5.8: Left apical aortic DE measurements in individual Dobermanns with DCM.

Dog	Ao vel ms <sup>-1</sup>	VTI cm	HR /min	dV/dt ms <sup>-2</sup>	acc dt s	ET s	PEP s	PEP/ET	IVRT s
1	0.82	6.50	137	19.11	0.046	0.142	0.095	0.669	0.017
2	0.77	5.73	172.7	20.5	0.040	0.143	0.067	0.469	0.006
3	1.22	11.64	134.1	29.99	0.045	0.156	0.091	0.583	0.008
4	0.83	7.99	133.7	14.69	0.060	0.164	0.090	0.549	0.015
5	0.89	7.74	159	15.37	0.060	0.150	0.084	0.560	0.006
6	1.11	11.30	89.5	39.6	0.028	0.150	0.122	0.813	0.002
7	0.83	7.73	151	12.22	0.070	0.171	0.083	0.485	0.018
8	0.66	7.33	105.6	12.93	0.056	0.190	0.105	0.553	0.024
9	0.63	4.22	163.9	16.73	0.039	0.109	0.093	0.853	0.011
10	0.62	5.05	187	15.38	0.042	0.141	NR	NR	NR
11	0.69	5.88	130.2	14.74	0.050	0.139	0.091	0.655	0.011
mean	0.82	7.37	142.15	19.21	0.05	0.150	0.092	0.62	0.012
SD	0.19	2.35	28.62	8.35	0.01	0.021	0.014	0.13	0.007

Ao vel: aortic velocity, VTI: velocity time integral, HR: heart rate, dV/dt: aortic flow acceleration, acc dt: aortic flow acceleration time, ET: left ventricular ejection time, PEP: left ventricular pre-ejection period, IVRT: isovolumic relaxation time, SD: standard deviation, NR: not recorded.



Table 5.9. Mitral inflow DE measurements in individual Dobermanns with DCM.

Dog	E vel ms <sup>-1</sup>	A vel ms <sup>-1</sup>	E/A	E DT s	E dur s	E decel ms <sup>-2</sup>
1	1.09	0.5	2.18	0.041	0.0115	31.83
2	1.13	0.6	1.85	0.057	0.088	21.6
3	1.11	0.43	2.58	0.037	0.066	32.84
4	0.76	0.41	1.32	0.060	0.12	11.91
5	0.91	0.44	2.07	0.070	0.1	NR
6	0.86	0.46	1.87	0.096	0.187	9.35
7	0.59	0.53	1.11	0.055	0.123	11.16
8	0.56	0.34	1.65	0.056	0.089	13.62
9	1.09	n/a	n/a	0.067	0.137	17.43
10	0.76	n/a	n/a	0.090	0.122	9.21
11	0.8	n/a	n/a	0.049	0.098	18.4
mean	0.88	0.46	1.83	0.062	0.10	17.74
SD	0.21	0.08	0.47	0.018	0.04	8.69

DE: Doppler echocardiography, E vel: velocity of early filling, A vel: velocity of atrial filling, E/A: ratio of early to atrial filling velocities, E DT: mitral E wave deceleration time, E dur: duration of mitral E wave, E decel: mitral E wave deceleration, SD: standard deviation, n/a: not applicable.

Table 5.10: Additional Doppler measurements in Dobermanns with DCM

Dog	PA		TV			TR		MR			
	vel ms <sup>-1</sup>	dV/dt ms <sup>-2</sup>	E vel ms <sup>-1</sup>	A vel ms <sup>-1</sup>	E/A	grade 0 - 4	vel ms <sup>-1</sup>	grade 0 - 4	vel ms <sup>-1</sup>	duration s	dP/dt mmHg s <sup>-1</sup>
1	0.37	6.47	0.42	0.5	0.84	1	NR	2	>4.00	0.260	1143
2	0.41	8.41	NR	NR	NR	NR	NR	NR	NR	NR	NR
3	0.62	7.77	0.55	0.47	1.05	1	NR	2	NR	0.270	1455
4	0.43	6.45	0.61	0.57	1.02	3	3.3	3	4.25	0.260	1185
5	0.45	5.01	0.89	0.42	2.12	0	-	1	NR	NR	NR
6	0.44	6.1	0.48	0.31	1.55	2	2.46	2	4.67	0.292	1143
7	0.55	9	0.28	0.16	1.75	2	2.27	2	4.16	0.300	1455
8	0.47	5.45	0.36	0.27	1.33	0	1.33	1	NR	NR	NR
9	0.36	8.38	0.59	n/a	n/a	2	NR	2	3.28	0.180	914
10	NR	NR	0.37	n/a	n/a	0	-	3	3.87	0.234	941
11	NR	NR	NR	NR	NR	0	-	2	3.80	0.229	941
mean	0.46	7.00	0.52	0.39	1.24	1.1 (1)	2.68	2.00 (2)	4.01	0.253	1147
SD	0.08	1.42	0.19	0.15	0.60	1.1	0.55	0.67	0.47	0.039	217

PA: pulmonary artery, TV: tricuspid valve, vel: velocity, dV/dt: acceleration, E vel: velocity of early filling, A vel: velocity of atrial filling, E/A: ratio of early to atrial filling velocities, TR: tricuspid regurgitation, grade: severity of tricuspid or mitral regurgitation (0 – 4), MR: mitral regurgitation, duration: duration of mitral regurgitation, dP/dt: rate of rise of left ventricular pressure calculated from mitral regurgitation signal, SD: standard deviation, n/a: not applicable, NR: not recorded. Median values expressed in brackets.

### 5.5.3 Cocker spaniels with DCM

Aortic DE variables for cocker spaniels with DCM are displayed in table 5.11, mitral inflow measurements are shown in table 5.12, and right heart variables and atrioventricular valve regurgitation measurements are listed in table 5.13.

Table 5.11: Left apical aortic DE measurements in individual Cocker spaniels with DCM

Dog	Ao vel ms <sup>-1</sup>	VTI cm	HR /min	dV/dt ms <sup>-2</sup>	acc dt s	ET s	PEP s	PEP/ET	IVRT s
1	0.65	4.05	167	16.84	0.042	0.152	0.091	0.599	0.055
2	0.76	5.17	177.4	18.81	0.047	0.128	0.078	0.609	0.026
3	0.75	5.2	180.44	21.09	0.038	0.124	0.104	0.839	0.06
4	0.95	6.72	170.2	24.14	0.043	0.147	0.088	0.599	0.023
5	0.96	7.76	157.6	27.47	0.038	0.137	0.096	0.701	0.035
6	0.92	8.63	110.22	17.92	0.058	0.175	0.116	0.663	0.049
7	0.68	5.55	141.1	19.64	0.04	0.148	0.102	0.689	0.0575
8	0.74	6.61	106.6	15.97	0.049	0.150	0.074	0.493	0.019
9	1.09	10.07	124.8	24.9	0.048	0.148	0.096	0.649	0.06
10	1.66	11.44	123.6	41	0.042	0.129	0.053	0.411	0.024
11	0.86	7.52	95.8	14.33	0.059	0.175	0.117	0.669	0.086
mean	0.91	7.16	141.34	22.01	0.05	0.147	0.092	0.63	0.04
SD	0.28	2.24	30.70	7.48	0.01	0.017	0.019	0.11	0.02

DE: Doppler echocardiography, Ao vel: aortic velocity, VTI: velocity time integral, HR: heart rate, dV/dt: aortic flow acceleration, acc dt: aortic flow acceleration time, ET: left ventricular ejection time, PEP: left ventricular pre-ejection period, IVRT: isovolumic relaxation time, SD: standard deviation.

Table 5.12: Mitral inflow DE measurements in individual Cocker spaniels with DCM

Dog	E vel ms <sup>-1</sup>	A vel ms <sup>-1</sup>	E/A	E DT s	E dur s	E dV/dt ms <sup>-2</sup>
1	0.29	0.96	0.30	0.068	0.098	4.52
2	1.43	n/a	n/a	0.043	0.088	38.95
3	0.91	0.61	1.47	0.064	0.091	14.78
4	1.06	n/a	n/a	0.067	0.128	16.67
5	0.34	0.29	0.95	0.097	0.184	4.24
6	0.61	0.87	0.70	0.077	0.115	8.28
7	0.77	0.52	1.48	0.065	0.102	13.25
8	0.87	0.6	1.45	0.079	0.127	11.33
9	1.08	0.88	1.23	0.060	0.1	19
10	1.21	0.66	1.83	0.068	0.093	18.43
11	0.66	0.54	1.22	0.078	0.116	8.75
mean	0.84	0.66	1.18	0.070	0.11	14.38
SD	0.35	0.21	0.47	0.014	0.03	9.62

DE: Doppler echocardiography, E vel: velocity of early filling, A vel: velocity of atrial filling, E/A: ratio of early to atrial filling velocities, E DT: mitral E wave deceleration time, E dur: duration of mitral E wave, E -dV/dt: mitral E wave deceleration, SD: standard deviation, n/a: not applicable.

Table 5.13: Additional DE measurements in Cocker spaniels with DCM

Dog	PA		TV			TR		MR			
	vel ms <sup>-1</sup>	dV/dt ms <sup>-2</sup>	E vel ms <sup>-1</sup>	A vel ms <sup>-1</sup>	E/A	Grade 0 – 4+	Vel ms <sup>-1</sup>	Grade 0 – 4+	vel ms <sup>-1</sup>	dur s	dP/dt mmHg s <sup>-1</sup>
1	0.52	7.44	0.95	n/a	n/a	2	NR	3	3.67	0.255	865
2	0.48	6.11	0.7	n/a	n/a	0	n/a	4	4.11	0.23	1231
3	0.43	5.95	0.46	n/a	n/a	1	1.5	2	4.28	0.268	800
4	0.58	10.26	0.85	n/a	n/a	0	n/a	2	3.93	0.251	1143
5	0.96	16.79	0.43	0.29	1.34	0	n/a	1	NR	0.28	711
6	0.65	6.55	0.58	0.66	0.87	2	2	3	4.88	0.402	478
7	0.55	7.44	0.46	0.48	0.96	2	2.52	3	4.84	0.31	865
8	0.52	8.08	0.32	0.16	2.00	2	2.4	4	4.63	0.26	1185
9	0.69	8.09	0.53	0.6	0.88	0	n/a	2	4.35	0.31	842
10	1.09	13.48	0.74	0.69	1.07	2	2.86	3	4.81	NR	970
11	0.59	5.84	NR	NR	NR	2	NR	2	4.27	0.354	533
mean	0.64	8.73	0.60	0.48	1.19	1.18 (2)	2.45	2.64 (3)	4.38	0.29	874.76
SD	0.20	3.49	0.20	0.21	0.43	0.98	0.35	0.92	0.41	0.05	247.37

DE: Doppler echocardiography, PA: pulmonary artery, vel: velocity, dV/dt: acceleration, TV: tricuspid valve, E vel: velocity of early filling, A vel: velocity of atrial filling, E/A: ratio of early to atrial filling velocities, TR: tricuspid regurgitation, grade: severity of tricuspid or mitral regurgitation (0 – 4), MR: mitral regurgitation, dur: duration of mitral regurgitation, dP/dt: rate of rise of left ventricular pressure calculated from mitral regurgitation signal, SD: standard deviation, n/a: not applicable, NR: not recorded. Median values expressed in brackets.

#### 5.5.4 Comparison of GSH pointers and dogs with DCM

A summary of the mean values for each group (normal GSH pointers, Dobermanns with DCM, and cocker spaniels with DCM) is displayed in table 5.14, with the results of comparisons between the groups. The raw data for all groups for all variables are displayed in graphs 5.1 to 5.24.

Differences between the three groups by one-way analysis of variance or Kruskal-Wallis one-way analysis of variance on ranks showed significant differences for all variables at a significance level of  $p < 0.001$  with the following exceptions: aortic acceleration ( $p = 0.032$ ); mitral A velocity ( $p = 0.006$ ); mitral E/A ratio ( $p =$

0.009); tricuspid E/A ratio ( $p = 0.038$ ). There were no significant differences for aortic acceleration time, mitral E velocity, or tricuspid E and A velocities. Blood flow velocities in the three groups of dogs are compared with previous reports in table 5.16.

Table 5.14: Group mean DE values for GSH pointers, Dobermanns and Cocker spaniels, with results of group comparisons.

Variable	units	GSPs		Dobes		Cockers		ANOVA P value	Significant pairwise differences (p<0.05)		
		mean	SD	mean	SD	mean	SD		Dob vs GSP	CSp vs GSP	Dob vs CSp
L. apex aortic vel	ms <sup>-1</sup>	1.24	0.19	0.83	0.19	0.91	0.28	<0.001	+	+	-
Ao VTI	cm	13.33	2.88	7.37	2.35	7.16	2.24	<0.001	+	+	-
HR	/min	83.6	16.05	142.2	28.62	141.3	30.7	<0.001	+	+	-
dV/dt	ms <sup>-2</sup>	27.28	6.05	19.21	8.35	22.01	7.48	<0.05	+	-	-
AT	s	0.050	0.006	0.049	0.012	0.046	0.007	NS	-	-	-
ET	s	0.198	0.012	0.150	0.0207	0.147	0.0171	<0.001	+	+	-
PEP	s	0.059	0.006	0.092	0.014	0.092	0.019	<0.001	+	+	-
PEP/ET	-	0.302	0.037	0.619	0.129	0.629	0.111	<0.001	+	+	-
PA vel	ms <sup>-1</sup>	0.90	0.172	0.46	0.083	0.61	0.19	<0.001	+	+	-
PA dV/dt	ms <sup>-2</sup>	12.32	3.20	7.00	1.43	7.92	2.36	<0.001	+	+	-
MV E vel	ms <sup>-1</sup>	0.69	0.12	0.88	0.21	0.84	0.35	NS	NS	NS	NS
MV A vel	ms <sup>-1</sup>	0.43	0.06	0.46	0.08	0.66	0.21	<0.01	+	+	-
MV E/A	-	1.55	0.286	1.83	0.471	1.18	0.466	<0.01	-	-	+
MV DT	s	0.086	0.011	0.062	0.018	0.070	0.013	<0.005	+	+	-
IVRT	s	0.036	0.021	0.012	0.007	0.045	0.021	<0.001	+	-	+
TV E vel	ms <sup>-1</sup>	0.59	0.13	0.52	0.19	0.60	0.20	NS	NS	NS	NS
TV A vel	ms <sup>-1</sup>	0.36	0.10	0.39	0.15	0.48	0.21	NS	NS	NS	NS
TV E/A	-	1.74	0.43	1.38	0.46	1.19	0.43	<0.05	-	+	-
MR	/4	n/a	n/a	2	(1 – 3)	3	(1 – 4)	NS	NS	NS	NS
MR dP/dt	mm/Hg	n/a	n/a	1147	217	875	247	t-test <0.05	n/a	n/a	+
MR vel	ms <sup>-1</sup>	n/a	n/a	4.01	0.47	4.38	0.41	t-test NS	-	-	-
TR	/4	n/a	n/a	1	(0 – 3)	2	(0 – 2)	t-test NS	NS	NS	NS

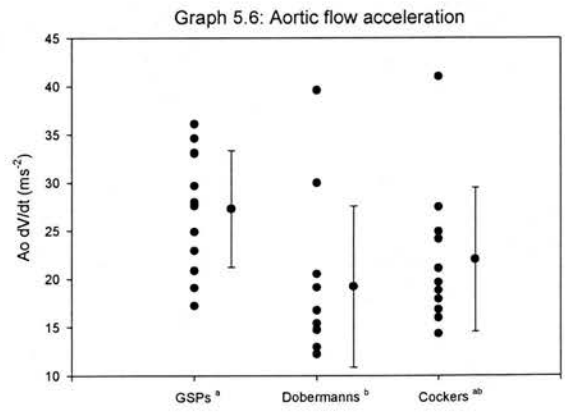
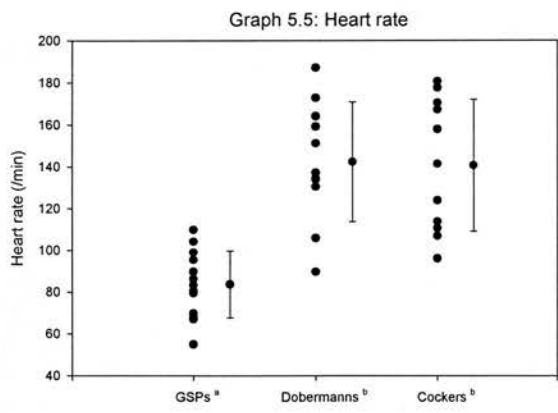
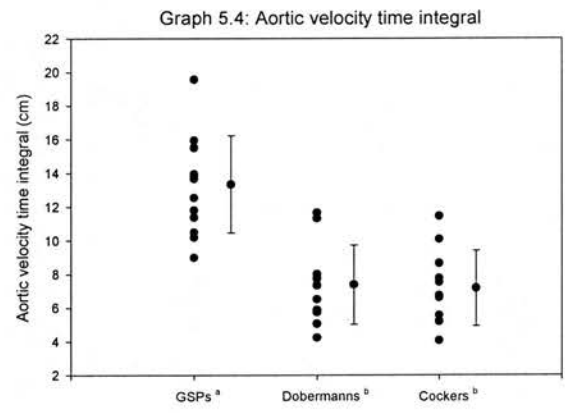
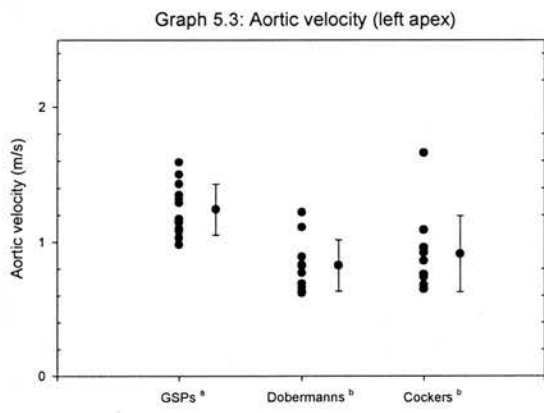
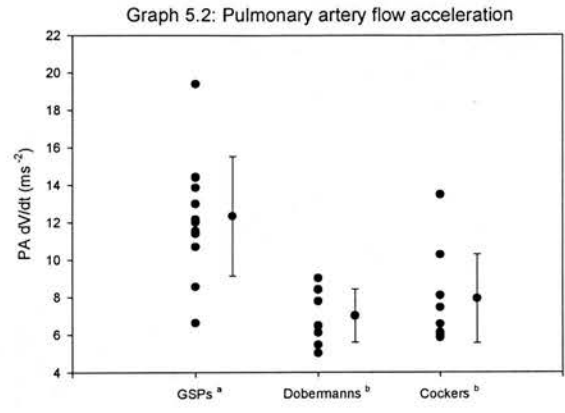
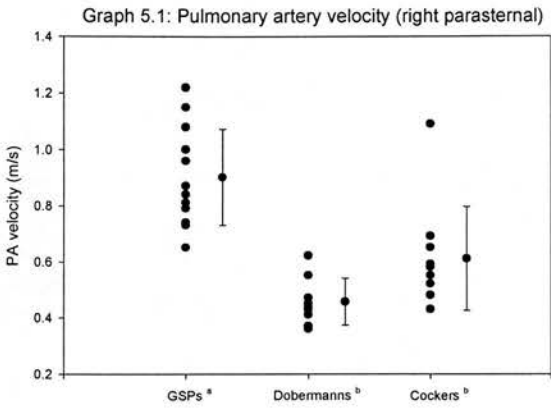
GSPs: German short-haired pointers, Dobes / Dob: Dobermanns, Cockers / CSp: cocker spaniels, ANOVA: analysis of variance, L. apex aortic vel: aortic velocity from left apical view, Ao VTI: aortic velocity time integral, HR: heart rate, dV/dt: aortic flow acceleration, AT: aortic flow acceleration time, ET: left ventricular ejection time, PEP: left ventricular pre-ejection period, PA vel: pulmonary artery velocity, PA dV/dt: pulmonary artery acceleration, MV: mitral valve, E vel: velocity of early filling, A vel: velocity of atrial filling, E/A: ratio of early to atrial filling velocities, MV DT: mitral E wave deceleration time, IVRT: isovolumic relaxation time, TV: tricuspid valve, MR: mitral regurgitation, MR dP/dt: rate of rise of left ventricular pressure calculated from mitral regurgitation signal, MR vel: velocity of mitral regurgitation, TR: tricuspid regurgitation, SD: standard deviation, NS: not significant, n/a: not applicable.

#### *5.5.4.1 Pulmonary artery and aortic variables*

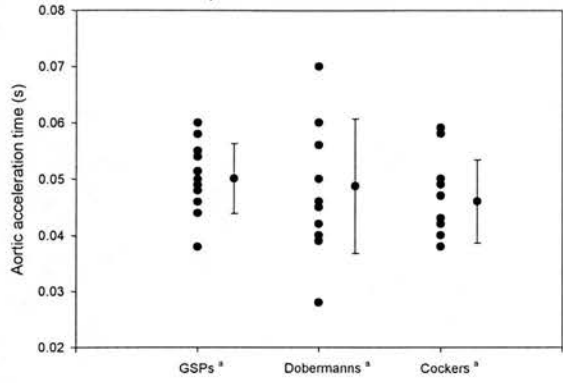
Pulmonary artery velocities (shown in graph 5.1) were higher ( $p < 0.05$ ) in the GSH pointers ( $0.90 \pm 0.172$  m/s, versus  $0.46 \pm 0.083$  m/s and  $0.61 \pm 0.19$  m/s in the Dobermanns and cockers, respectively). Pulmonary artery acceleration (graph 5.2) was also higher ( $p < 0.05$ ) in the pointers. Left apical velocities (graph 5.3) were significantly lower ( $p < 0.05$ ) in the DCM dogs (Dobermanns  $0.83 \pm 0.19$  m/s and cockers  $0.91 \pm 0.28$  m/s, versus  $1.24 \pm 0.19$  m/s in the normal dogs). Aortic velocity time integrals (graph 5.4) and aortic acceleration (graph 5.6) were also higher ( $p < 0.05$ ) in the normal dogs; however, aortic acceleration time was similar in all three groups.



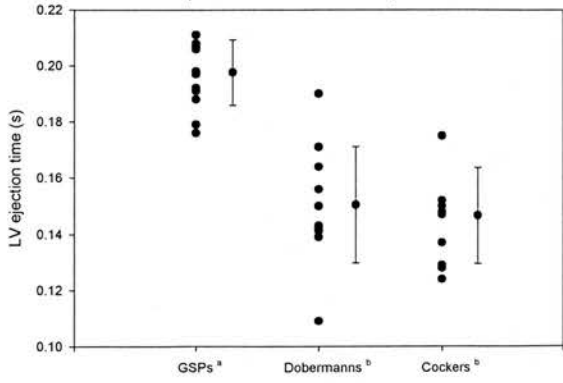
COMPARISON OF DOPPLER VARIABLES IN ALL GROUPS



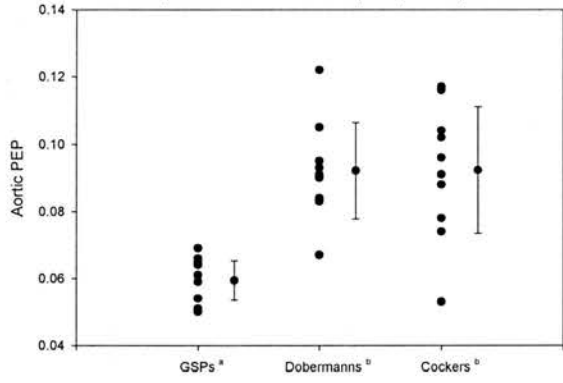
Graph 5.7: Aortic acceleration time



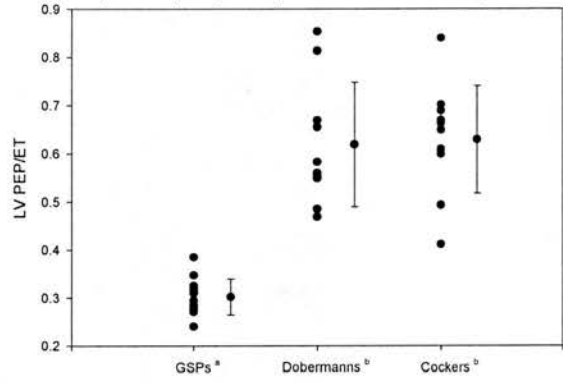
Graph 5.8: Left ventricular ejection time



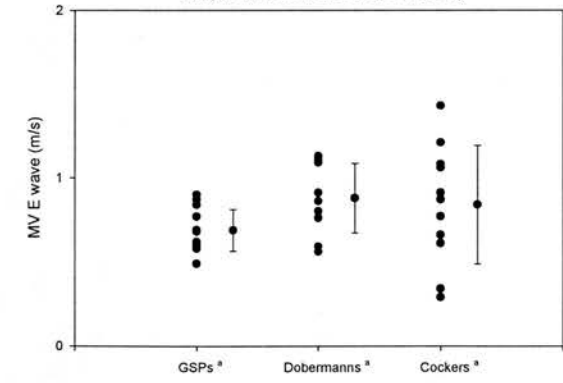
Graph 5.9: Left ventricular pre-ejection period



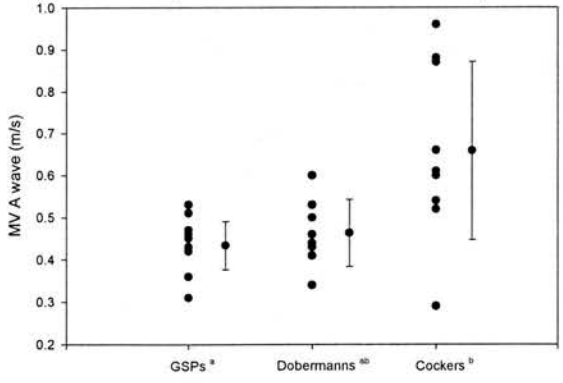
Graph 5.10: pre-ejection period: left ventricular ejection time



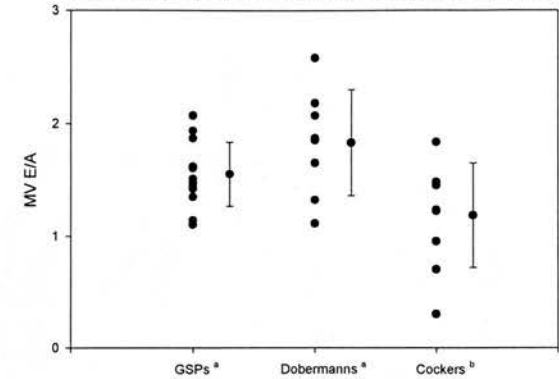
Graph 5.11: Mitral E wave velocity



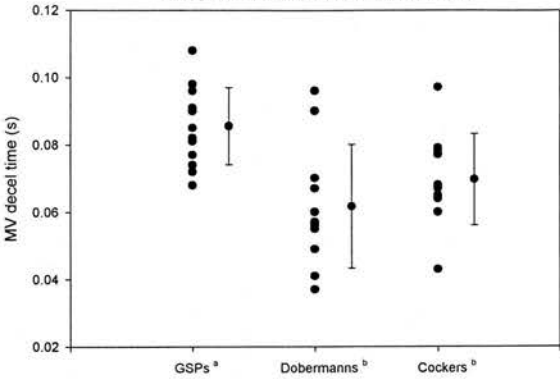
Graph 5.12: Mitral valve A wave velocity



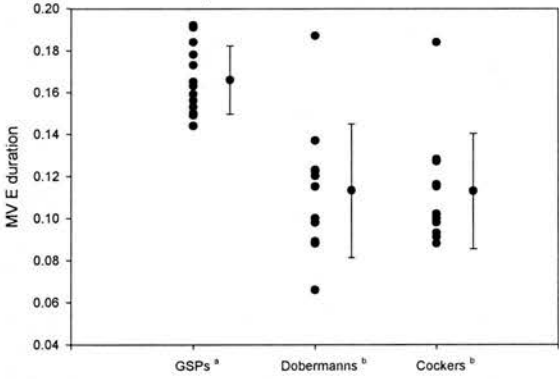
Graph 5.13: Transmitral flow ratio of early : atrial filling (E/A)



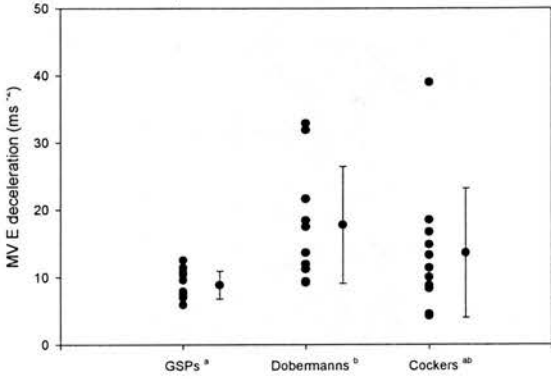
Graph 5.14: Mitral deceleration time



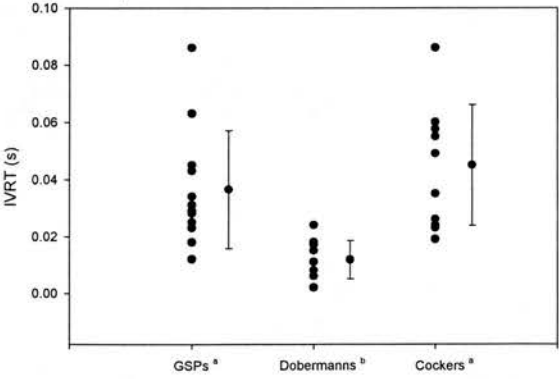
Graph 5.15: Mitral E wave duration



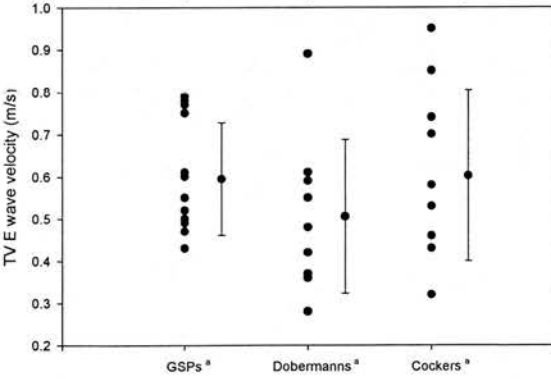
Graph 5.16: Mitral E wave deceleration



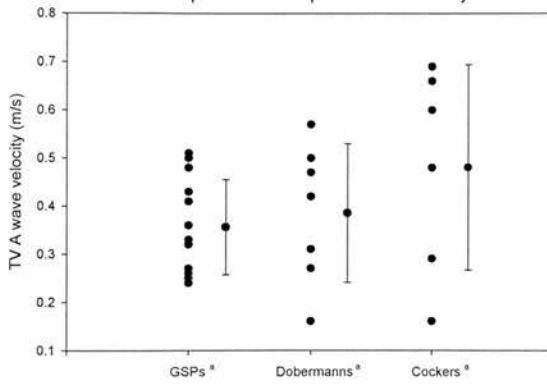
Graph 5.17: Left ventricular isovolumic relaxation time



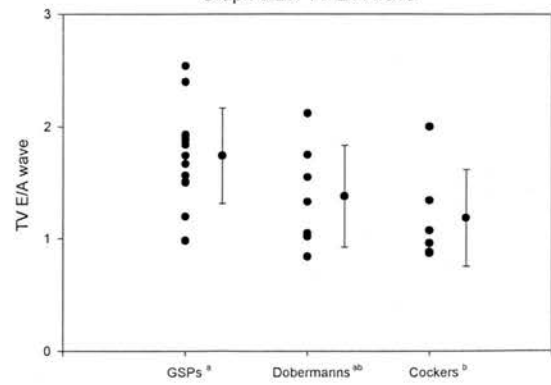
Graph 5.18: Tricuspid E wave velocity



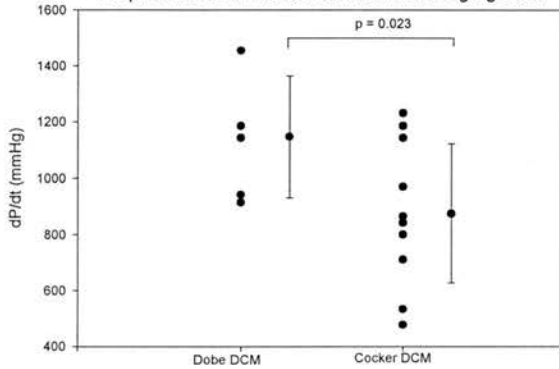
Graph 5.19: tricuspid A wave velocity



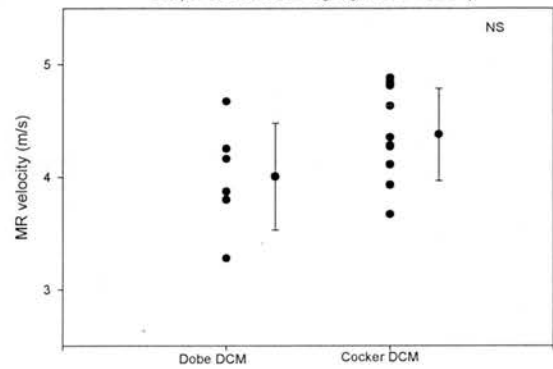
Graph 5.20: TV E/A wave



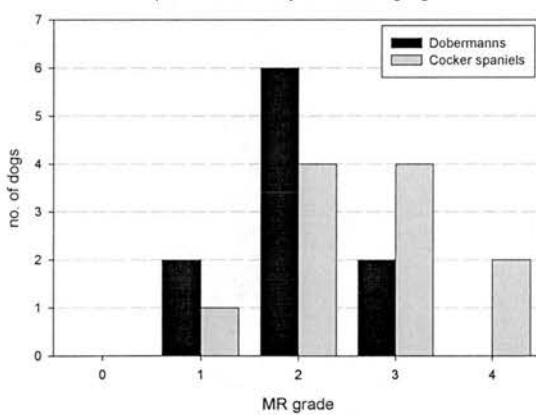
Graph 5.21: LV dP/dt derived from mitral regurgitation



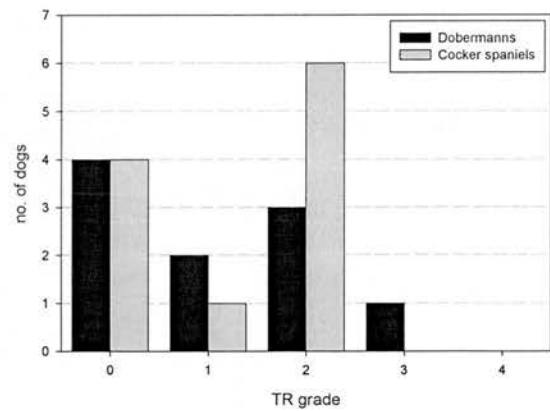
Graph 5.22: Mitral regurgitation velocity



Graph 5.23: Severity of mitral regurgitation



Graph 5.24: Severity of tricuspid regurgitation



When German short-haired pointers (GSPs), Dobermanns or English cocker spaniels (cockers) were statistically different following analysis of variance, a different letter in superscript is ascribed (eg. GSP<sup>a</sup> and Dobermann<sup>b</sup> indicates the two groups were significantly different at the  $p < 0.05$  level in pairwise comparisons). GSPs: German short-haired pointers, LV: left ventricle, dP/dt: rate of change of left ventricular pressure, E: early filling, A: atrial filling.

#### *5.5.4.2 Systolic time intervals*

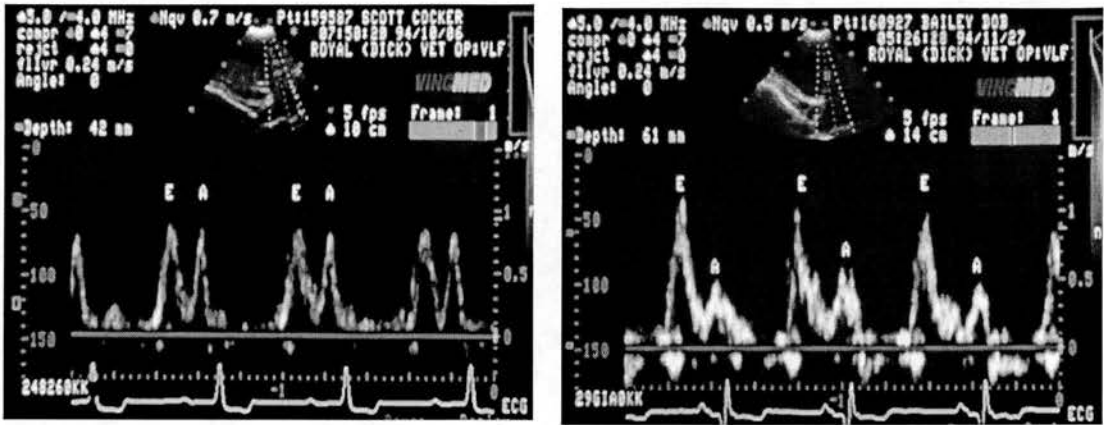
The systolic time intervals were clearly different in the normal and DCM groups. Pre-ejection period (PEP, graph 5.9) was shorter ( $p < 0.05$ ) in the normal dogs ( $0.059 \pm 0.006$  sec, versus  $0.092 \pm 0.014$  sec in the Dobermanns, and  $0.092 \pm 0.019$  sec in the cockers). Ejection time (ET, graph 5.8) was longer ( $p < 0.05$ ) in the normal dogs ( $0.198 \pm 0.012$  sec in the pointers,  $0.15 \pm 0.021$  sec in the Dobermanns and  $0.147 \pm 0.017$  sec in the cockers). The ratio of these, PEP/LVET (graph 5.10) was lower ( $p < 0.05$ ) in the normal dogs ( $0.302 \pm 0.037$ ) than in the DCM dogs ( $0.619 \pm 0.129$  for the Dobermanns,  $0.629 \pm 0.111$  for the cockers).

#### *5.5.4.3 Transmitral filling*

There were no significant differences in mitral E wave velocity (graph 5.11), although there was a tendency towards higher values in the DCM dogs ( $0.88 \pm 0.21$  m/s and  $0.84 \pm 0.35$  m/s, compared with  $0.69 \pm 0.12$  m/s in the pointers). Although the cockers had the highest values for mitral A wave velocity (graph 5.12), their values were widely spread ( $0.66 \pm 0.21$  m/s) with no significant difference compared to the Dobermanns ( $0.46 \pm 0.08$  m/s), and significance only being reached when compared with the normal dogs ( $0.43 \pm 0.06$  m/s,  $p < 0.05$ ). However, there were significant differences ( $p < 0.05$ ) between the cockers and Dobermanns in mitral E/A ratio (graph 5.13). Dobermanns had the highest value ( $1.83 \pm 0.47$ ), and cockers the lowest ( $1.18 \pm 0.47$ ), with the normal dogs in-between ( $1.55 \pm 0.29$ ). Examples of transmitral flow from a Dobermann and a cocker spaniel are given in figure 5.2. The two cocker spaniels with a single transmitral wave were omitted

from the E/A analysis; these two dogs had high heart rates and it was suspected that the E and A waves were fused. Mitral deceleration times (Graph 5.14) were shorter ( $p < 0.05$ ) in the DCM dogs than in the normal dogs ( $0.086 \pm 0.011$ ), but no differences were found between the Dobermanns ( $0.062 \pm 0.018$ ) and the cockers ( $0.070 \pm 0.013$ ). Isovolumic relaxation time (Graph 5.17) was shortest ( $p < 0.05$ ) in the Dobermanns ( $0.012 \pm 0.007$  sec) and longest in the cockers ( $0.045 \pm 0.021$  sec), with significant differences between the two breeds. The normal pointers had an intermediate value ( $0.036 \pm 0.021$ ).

Figure 5.2: Spectral Doppler recordings of transmitral flow.



Transmitral flow showing early filling (E) and atrial filling (A) waves. The recording on the left is from cocker 6, and shows almost equal velocities of the E and A waves. The recording on the right is from Dobermann 6, and shows an increased E/A ratio.

The transmitral filling patterns can be classified by combining the results of mitral E/A ratio, mitral deceleration time and isovolumic relaxation time (table 5.15). A restrictive filling pattern was defined as either a mitral E/A  $> 2.0$ , or a mitral E/A  $< 2.0$  but  $> 1.0$ , together with a mitral deceleration time  $\leq 0.070$  sec and an isovolumic relaxation time  $< 0.036$  sec. An abnormal relaxation pattern was defined as a mitral E/A  $< 1.0$ , or a mitral E/A  $> 1.0$  but  $< 2.0$ , together with a mitral deceleration time  $> 0.070$  sec and an isovolumic relaxation time  $> 0.036$  sec. Pseudonormal patterns were defined as a mitral E/A  $> 1.0$  but  $< 2.0$ , with only one of the two other variables shorter than normal. According to this classification, 7/8 of the Dobermanns in sinus rhythm had a restrictive pattern, compared to only 3/11 cocker spaniels. None of the Dobermanns showed a delayed relaxation pattern, compared with four of the cocker spaniels. One Dobermann showed pseudonormal filling, in comparison with 4/11 of the cocker spaniels. In terms of transmitral filling, the cocker spaniels appeared to be a more heterogeneous group.



Table 5.15: Transmitral flow patterns in Dobermanns and cocker spaniels with DCM

Dobermanns					Cocker spaniels				
Dog	E/A	E DT	IVRT	Filling pattern	Dog	E/A	E DT	IVRT	Filling pattern
	> 2.0	≤ 0.070 sec	< 0.036 sec			< 1.0	≤ 0.070 sec	< 0.036 sec	
1	res	+	+	restrictive	1	ab	+	-	abnormal relaxation
2	-	+	+	restrictive	2	fused	+	+	restrictive
3	res	+	+	restrictive	3	-	+	-	pseudonormal
4	-	+	+	restrictive	4	fused	+	+	restrictive
5	res	+	+	restrictive	5	ab	-	+	abnormal relaxation
6	-	-	+	pseudonormal	6	ab	-	-	abnormal relaxation
7	-	+	+	restrictive	7	-	+	-	pseudonormal
8	-	+	+	restrictive	8	-	-	+	pseudonormal
9	AF	+	+	-	9	-	+	-	pseudonormal
10	AF	-	NR	-	10	-	+	+	restrictive
11	AF	+	+	-	11	-	-	-	abnormal relaxation

A **restrictive** filling pattern was defined as either a mitral E/A > 2.0, or a mitral E/A < 2.0 but > 1.0, together with a mitral deceleration time ≤ 0.070 sec and an isovolumic relaxation time < 0.036 sec. An **abnormal relaxation** pattern was defined as a mitral E/A < 1.0, or a mitral E/A > 1.0 but < 2.0, together with a mitral deceleration time > 0.070 sec and an isovolumic relaxation time > 0.036 sec. Pseudonormal patterns were defined as a mitral E/A > 1.0 but < 2.0, with one of the two other variables shorter than normal.

E/A = ratio of mitral early (E) to late (A) peak flow velocities; E DT = mitral E wave deceleration time; IVRT = isovolumic relaxation time; res = restrictive; AF = atrial fibrillation, ab = abnormal relaxation.

#### 5.5.4.4 Tricuspid inflow

No significant differences were found in tricuspid velocities (graphs 5.18, 5.19) between the three groups, although the cockers had significantly lower tricuspid E/A ratios ( $1.19 \pm 0.43$ ) compared with the normal dogs ( $1.74 \pm 0.43$ , graph 5.20). The Dobermanns also had lower values for tricuspid E/A than the normal dogs ( $1.38 \pm 0.46$ ) but did not differ significantly from either of the other two groups.

#### 5.5.4.5 Mitral and tricuspid regurgitation

One of the GSH pointers had trivial tricuspid regurgitation, but none of the normal dogs had mitral regurgitation. The rate of left ventricular pressure development (LV dP/dt) calculated from the spectral signal of mitral regurgitation was  $1147 \pm 217$  mmHg/sec for the Dobermanns, and  $875 \pm 247$  mmHg/sec in the cockers, with the latter group being significantly lower (graph 5.21). The velocity of mitral regurgitation was not significantly different in the two breeds (graph 5.22), although it was slightly higher in the cockers ( $4.38 \pm 0.41$  m/s, versus  $4.01 \pm 0.47$  m/s in the Dobermanns). On a scale of 1 (trivial) to 4 (severe), the median score of mitral regurgitation for Dobermanns was 2, and 3 for cocker spaniels, although the difference did not reach significance. A histogram of the distribution of grades in the two breeds is shown in graph 5.23. Using the same scale as with mitral regurgitation, there was no significant difference in the degree of tricuspid regurgitation between the two DCM groups, although the median grade in Dobermanns was 1, and the median grade in the cockers was 2. The grade distribution by breed for tricuspid regurgitation is shown in graph 5.24.

## 5.6 DISCUSSION

Applications of DE have become increasingly sophisticated over the past fifteen years, evolving from its original use as a simple aortic flow velocimeter (Light, 1969), to a tool that can be used to derive purer indices of cardiac function such as  $\tau$  (Scalia *et al.*, 1997) and LV dP/dt (Chung *et al.*, 1992). Formerly, these measurements were restricted to the research catheterisation laboratory. In veterinary cardiology there has been a tendency to regard DE as most useful in the

assessment of congenital cardiac defects and valvular disease, and to rely on M-mode indices for assessment of ventricular function. No doubt this reflects gross under-usage of DE, but with an ever-expanding number of DE variables to measure and calculate, it is essential to have a proper understanding of the strengths and limitations of each variable in order to apply them in a discriminating manner. In this part of the study, a wide range of DE variables were used in normal dogs and dogs with DCM to evaluate their repeatability, and their ability to discriminate between normal cardiac function and myocardial failure. Furthermore, differences were sought between the two breeds of dog affected with DCM, to highlight any variables that might be worth pursuing as prognostic indicators.

#### 5.6.1 Normal GSH pointers

Values in the normal GSH pointers for all the velocities across all the valves are comparable with those previously reported (see table 5.16) if one excludes Kirberger's angle-corrected results which probably over-estimate velocities (Kirberger *et al.*, 1992). Velocities from the left apical view were lower than for the subcostal view, as has been reported by several others (Lehmkuhl and Bonagura, 1994). Despite the fact that the subcostal view is often advocated for measuring aortic velocities when screening for aortic stenosis, it is difficult to find published normal values for aortic velocities from this view in the dog. The increased depth of field required for this view often results in weaker signals, and aliasing is a problem without HPRF systems. In addition, transducer placement often requires firm pressure to maintain alignment of the probe, which is resented by many unsedated dogs. For many of the DCM dogs in this study, poor patient co-operation was the

main reason for the difficulties in obtaining subcostal signals. As a result, left apical views are often accepted despite inferior alignment with flow.

The systolic time intervals in the normal dogs fall in the mid-range of values reported in the literature. In this study, the mean PEP/LVET value in the GSH pointers was  $0.30 \pm 0.04$ , compared with the relatively low values (0.24) reported by Amberger and Lombard (1998) and high values ( $0.44 \pm 0.01$ ) reported by Minors and O'Grady (1998) in "normal" Dobermanns. If one excludes values obtained in Dobermanns or other breeds predisposed to dilated cardiomyopathy, the highest PEP/LVET reported for conscious, unsedated dogs is  $0.38 \pm 0.06$  from greyhounds (Snyder *et al.*, 1995a). Dukes McEwan reported values of PEP/LVET in normal Newfoundlands of  $0.63 \pm 0.05$  (Dukes McEwan, 1998a), but these studies were recorded with an Esaote Challenge 7000 machine, which has since been identified as having time calibration faults in some machines, resulting in unreliable PEP/LVET ratios (Amberger and Lombard, 1998). Source details are not listed for the normal value of 0.24 referenced in Amberger and Lombard's study, although a value of  $0.29 \pm 0.04$  is given for their own study in normal small breed dogs. Pipers and others (1978) originally reported a value of  $0.24 \pm 0.09$  in anaesthetised dogs, and this may be the origin of the quoted value (Pipers *et al.*, 1978). Excluding the variables from anaesthetised animals and breeds at risk of occult DCM gives a much tighter normal range of 0.28 – 0.39.

Table 5.16: DE velocities in canine studies

	n	Aorta ms <sup>-1</sup>	Ao VTI cm	Ao dV/dt	PA ms <sup>-1</sup>	MV (E) ms <sup>-1</sup>	MV (A) ms <sup>-1</sup>	MV E/A	TV (E) ms <sup>-1</sup>	TV (A) ms <sup>-1</sup>
Gaber 1987	28	1.18 ± 0.18	-	-	1.00 ± 0.31	0.75 ± 0.12	0.54 ± 0.87	1.4 ± 0.2	-	-
Vandenberg <i>et al.</i> 1990 (young)	5	-	-	-	-	0.60 ± 0.16	0.32 ± 0.05	1.72	-	-
Vandenberg <i>et al.</i> 1990 (old)	6	-	-	-	-	0.49 ± 0.09	0.37 ± 0.06	1.27	-	-
Yuill & O'Grady 1991	20	1.18 ± 0.11	-	-	0.98 ± 0.94	0.86 ± 0.95	-	-	0.69 ± 0.84	-
Brown & Knight 1991	28	1.06 ± 0.21	13.1 ± 2.8	-	0.84 ± 0.17	-	-	-	-	-
Kirberger <i>et al.</i> 1992	50	1.57 ± 0.33	-	-	1.20 ± 0.20	0.91 ± 0.15	0.63 ± 0.13	1.48 ± 0.31	0.86 ± 0.20	0.58 ± 0.16
Darke <i>et al.</i> 1993 (healthy)	20	1.19 ± 0.24	-	33.69 ± 15.58	0.99 ± 0.22	0.65 ± 0.18	0.43 ± 0.13	1.55 ± 0.36	0.57 ± 0.15	0.37 ± 0.15
Minors & O'Grady 1998 (healthy Dobes)	23	1.32 ± 0.05	15.6 ± 0.56	28.67 ± 1.62	-	0.75 ± 0.03	0.58 ± 0.03	1.35 ± 0.05	-	-
Sottiaux & Amberger 1998	32	1.03 ± 0.26	-	-	0.72 ± 0.12	-	0.62 ± 0.03	-	-	-
Pietra <i>et al.</i> 1998	20	1.09 ± 0.18	-	-	0.78 ± 0.19	0.76 ± 0.12	0.57 ± 0.12	1.33	0.67 ± 0.21	0.50 ± 0.16
Bonagura <i>et al.</i> 1998	15	1.15 ± 0.15	-	-	1.07 ± 0.14	0.74 ± 0.09	0.46 ± 0.11	1.61	0.60 ± 0.08	0.45 ± 0.07
GSPs	13	1.21 ± 0.18	12.8 ± 2.9	26.65 ± 5.75	0.90 ± 0.15	0.69 ± 0.13	0.43 ± 0.06	1.58 ± 0.31	0.60 ± 0.14	0.34 ± 0.1
Minors & O'Grady 1998 (occult DCM Dobes)	12	1.26 ± 0.06	14.7 ± 0.86	27.43 ± 0.95	-	0.81 ± 0.05	0.67 ± 0.04	1.23 ± 0.07	-	-
Darke <i>et al.</i> 1993 (DCM)	22	0.99 ± 0.23	-	21.63 ± 8.60	0.70 ± 0.18	0.83 ± 0.29	0.43 ± 0.24	2.26 ± 0.94	0.54 ± 0.20	0.35 ± 0.17
DCM Dobermanns (present study)	11	0.89 ± 0.18	8.25 ± 2.13	20.55 ± 9.58	0.47 ± 0.08	0.88 ± 0.23	0.46 ± 0.08	1.83 ± 0.47	0.51 ± 0.2	0.39 ± 0.14
DCM Cocker (present study)	11	0.80 ± 0.12	6.21 ± 1.51	20.24 ± 3.89	0.53 ± 0.07	0.79 ± 0.38	0.64 ± 0.24	1.06 ± 0.49	0.59 ± 0.22	0.40 ± 0.22

VTI = velocity time integral, Ao dV/dt: aortic acceleration, PA = pulmonary artery, MV = mitral valve, (E) = early filling, (A) = atrial filling, TV = tricuspid valve, GSP = German short-haired pointer, Dobes = Dobermanns, Cocker = cocker spaniels, DCM: dilated cardiomyopathy.

#### 5.6.1.1 Isovolumic relaxation times

The values for isovolumic relaxation time (IVRT) from the left apical recordings in the normal dogs were low compared with previous reports, although this was not true of the subcostal values (Schober *et al.*, 1998). Low values of IVRT have been associated with younger age in people (Abinader and Sharif, 1992), but the cause of the lower values in the left apical recordings is more likely to be an error in measurement technique for the interval from the Q wave of the ECG to aortic valve closure. This was measured directly for all subcostal aortic recordings, but was calculated as the sum of PEP and LVET for the left apical aortic data set. The addition of an extra measurement step may well have introduced additional error.

Ideally, IVRT should be measured in one step, from a 100 mm/sec sweep recording with a large pulsed wave sample volume between the left ventricular inlet and outlet, from the aortic valve closure artefact to the onset of mitral flow (Rakowski *et al.*, 1996). The limitation of the slow sweep speed on the Vingmed machine should be equally applicable to both sets of data, and is not likely to be a factor in the difference. Calculation of IVRT from Doppler recordings may overestimate rather than underestimate IVRT as measured by M-mode echocardiography, as the mitral valve cusp separation on M-mode occurs before the mitral valve opening artefact by Doppler (Lee *et al.*, 1990).

#### 5.6.2 Reproducibility

The coefficients of variation (CV%) were high (>15%) for IVRT calculated from both views, and from both dogs used in the repeatability studies. As mentioned above, repeatability might have been improved with higher sweep speeds, and a one-

step measurement technique. The CV% values for the other variables were also higher than for M-mode and 2DE. The reproducibility of aortic blood flow variables has been reported for human subjects, and has generally been found to be better for peak velocity, ejection time and velocity time integral than for acceleration time or cardiac output (Gardin *et al.*, 1984; Moulinier *et al.*, 1991). The work by Gardin and co-workers (1984) suggested a change in aortic peak flow velocity of more than 13% on serial recordings (recorded and measured by the same person) would represent a genuine change (Gardin *et al.*, 1984). However, heart rate variability can influence stroke distance and cardiac output calculations, and represents biological variability. Sinus arrhythmia is more prevalent in dogs than humans, and may influence measurements dependent on stroke volume unless many beats are averaged. In this study, averaging ten beats would be expected to have minimised the effects of sinus arrhythmia.

Another feature that may influence day-to-day variability in dogs is the effect of increasing familiarity with the echocardiographic procedure. Heart rate, pulmonary artery and subcostal aortic velocities tended to be higher for the first study and decreased with subsequent studies, as the dogs became accustomed to the procedure. Both dogs had been scanned previously, but it might still have been better to omit the first day's results. High levels of sympathetic tone can mimic the effect of exogenous administration of catecholamines, which is a standard form of "stress echo" test (Minors and O'Grady, 1998). Of the diastolic variables, excluding IVRT, the least repeatable variables were mitral E deceleration and mitral E deceleration time (see table 5.7). These variables have also been found to be the



least reproducible in humans, with percent precision varying from 10.1 to 19.5% in the Framingham study (Galderisi *et al.*, 1992).

### 5.6.3 Dobermanns and cockers spaniels with DCM

#### 5.6.3.1 Aortic and pulmonary artery variables

As found in the study by Darke and others (1993), the aortic velocities were significantly depressed in the DCM dogs compared with normal (Darke *et al.*, 1993), even without normalising the values by the ratio of the mid-left ventricle to aortic cross-sectional area, as suggested by Isaaz and others (1990) (Isaaz *et al.*, 1990). Despite this, differences in peak aortic velocity were not found by Minors and O'Grady (1998) between apparently normal Dobermanns, and Dobermanns with occult DCM (Minors and O'Grady, 1998). Similarly, although significant differences in mean aortic acceleration were found between normals and dogs with DCM in this study and the study by Darke and others, Minors and O'Grady found no significant reduction of aortic  $dV/dt$  in Dobermanns with occult DCM (Darke *et al.*, 1993; Minors and O'Grady, 1998). The pattern was similar with aortic velocity time integral (or stroke distance), although this was not reported by Darke and others (1993). It was decided to measure stroke distance rather than calculate stroke volume, as this avoided unnecessary error from two-dimensional aortic measurements. In retrospect, it would have been useful to compare stroke volume or stroke volume index between the groups of dogs. However, these were already available from the 2DE volumetric measurements.

Although pulmonary artery velocities have received very little attention in DCM (human or canine), both peak velocities and acceleration were significantly



reduced in the DCM dogs, as previously reported by Darke and others (Darke *et al.*, 1993). This probably reflects poor right ventricular systolic function, despite the fact that both Dobermanns and cocker spaniels generally show a predominance of left-sided signs of failure. Right ventricular systolic time intervals might have been valuable in evaluating right ventricular performance.

#### *5.6.3.2 Systolic time intervals*

The systolic time intervals PEP/LVET were unequivocally high in the DCM dogs in this study, although the values for PEP/LVET quoted by Amberger and Lombard (1998) for DCM dogs are below the range often quoted for normal dogs (Amberger and Lombard, 1998). The values quoted for normal Dobermanns by Minors and O'Grady (1998) are much higher than those quoted by other authors ( $0.44 \pm 0.013$ ), and as with other systolic variables in this breed, it is difficult to know whether this reflects normal breed variation or genuine systolic dysfunction. Values for PEP/LVET in Dobermanns that progressed to occult DCM were significantly higher at baseline than in the healthy Dobermanns in the Minors and O'Grady's study ( $0.53 \pm 0.02$ ), approaching the values described for the DCM dogs in the present study. This suggests that PEP/LVET might be a clinically useful index for screening, although the doubt cast on reliability of time calibration in Esaote echocardiographs should lead one to be circumspect when reviewing results derived from these machines (Amberger and Lombard, 1998).

#### 5.6.3.3 Transmitral flow

Although there was no difference between the affected Dobermanns and cocker spaniels for any of the systolic DE variables, there were differences evident amongst the diastolic measurements. This was not the case for mitral E wave velocities, however: these were similar in both normal and affected groups, in contrast with Darke's study, where DCM affected dogs had increased E wave velocities (Darke *et al.*, 1993). No differences were seen in mitral E wave velocity between normal Dobermanns and those with occult DCM in the Minors and O'Grady study (1998).

#### 5.6.3.4 Mitral E/A ratio

There were significant differences in the mitral E/A ratio between the two affected breeds, with the Dobermanns having a mitral E/A greater than the normal dogs, and the cockers having a mitral E/A lower than normal. A one-way analysis of variance did not show any significant differences between the values in normal dogs and the previously reported mitral E/A values listed in Table 5.16. The mitral E/A ratio in the affected cockers was significantly lower ( $p < 0.05$ ) than all the published values listed, except for the normal Dobermanns reported by Minors and O'Grady (1998). The affected Dobermann mitral E/A values in this study were significantly higher ( $p < 0.05$ ) than in the previous reports listed, except for the study by Darke and others (1993), and the GSH pointers in this study.

There is a well-documented influence of age on transmitral filling variables in people, with the mitral E/A ratio decreasing with increasing age (Kitzman *et al.*, 1991; Klein *et al.*, 1994; Voutilainen *et al.*, 1994). The influence of age on canine

transmitral filling variables has not been well studied, although one report suggests increasing age may affect mitral E/A ratio in a similar manner to people (Vandenberg *et al.*, 1990). However, there was no significant difference in age between the groups in the present study (chapter 2).

A plausible explanation is that the cockers had abnormal relaxation, but lower left atrial pressures and/or better ventricular compliance than the Dobermanns. In human subjects with DCM, most or all will have abnormal relaxation, but only those with low left atrial pressures or less severely affected compliance have reduced E/A ratios, and it is this group of patients that generally has improved survival (Lavine and Arends, 1989; Shen *et al.*, 1992).

The three Dobermanns with atrial fibrillation were obviously excluded from the analysis of E/A ratios, owing to the absence of an A wave in these dogs. Two of the cocker spaniels also had only one mitral filling wave, and were also excluded from the analysis. It was assumed that the E and A waves were fused in these individuals, as their heart rates were 177/min and 170/min, respectively. An alternative explanation is that these two dogs had an extreme form of restrictive pattern, without a measurable A wave. At slow heart rates, when a single filling wave occurs demonstrably before the P wave of the ECG, it is much easier to be confident about this type of pattern being restrictive. When a single filling wave occurs at or just after the P wave (as in these two cases), it is more likely that E-A fusion has occurred. Appleton and others (1991) showed that the heart rate at which E-A fusion occurs in normal, sedated dogs depends on the method of heart rate stimulation (Appleton *et al.*, 1991). Fusion occurred at higher heart rates ( $221 \pm 12$  bpm) when exogenous catecholamines were used as the method of heart rate

stimulation, compared with right atrial pacing. Isoproterenol administration was associated with a concomitant improvement in ventricular relaxation, demonstrated by a decrease in  $\tau$ . In dogs with DCM, one would expect delayed relaxation and abnormal chamber compliance, which would be likely to result in fusion of E and A waves at lower heart rates than in normal dogs.

#### *5.6.3.5 Mitral E wave deceleration time*

Mitral E wave deceleration times have not been widely reported in dogs. Schober and others (1998) reported values of  $0.081 \pm 0.017$  sec in normal dogs, which was similar to the findings in the present study (unsurprisingly, as that study comprised several normal GSH pointers from the present study). Both cockers and Dobermanns had shorter deceleration times than normal, but were not different from each other. This suggests that both groups had increased filling pressures, and/or decreased ventricular compliance compared to the normal dogs. Nishimura and others (1989) measured much longer mitral deceleration times ( $0.131 \pm 0.018$  sec) in dogs anaesthetised with pentobarbitone. Peak mitral E and A velocities were low in that study (mean E = 0.39 m/s, mean A = 0.18 m/s) and the mean mitral E/A ratio was 2.5, suggesting that values obtained in anaesthetised animals should not be directly compared with those obtained in conscious, unsedated dogs (Nishimura *et al.*, 1989). Appleton's group (1994) measured transmitral variables in dogs lightly sedated with hydromorphone and diazepam, (following halothane anaesthesia) and found deceleration times were shorter ( $0.121 \pm 0.018$ ), but still longer than in this study and the study by Schober and others (1998) (Appleton *et al.*, 1994).

Care should be taken when comparing animals in sinus rhythm with animals in atrial fibrillation, as variations in cycle length may influence mitral deceleration time (Oh *et al.*, 1999a). Mitral deceleration time is probably one of the transmitral Doppler flow variables that is most readily applied to individuals with atrial fibrillation, and it appears to have similar prognostic value to that seen in patients in sinus rhythm (Hurrell *et al.*, 1998). Excluding the Dobermanns with atrial fibrillation from the statistical analysis did not alter the outcome.

#### *5.6.3.6 Isovolumic relaxation time*

The isovolumic relaxation times were lowest in the affected Dobermanns, with the cockers having significantly prolonged IVRT when compared with the Dobermanns. Omitting the Dobermanns with atrial fibrillation did not change the results of the statistical analysis.

Unfortunately, the left apical IVRT values were measured differently in the GSH pointers (using formula 5.2, instead of formula 5.1, as used in the dogs with DCM). It is therefore difficult to know whether the cockers had IVRT values that were genuinely higher than in normal dogs. If one compares the mean cocker IVRT (from the left apical view) with the IVRT measured from the subcostal recordings in the normal dogs, then the cockers had shortened isovolumic relaxation times. If one uses the left apical recordings from the normal dogs, the cockers had prolonged isovolumic relaxation times compared to normal. Again, there is little available in the literature on normal isovolumic relaxation times in conscious dogs to help resolve this issue. Unpublished data collected subsequent to this study suggest that

all the IVRT values recorded by this indirect method are shorter than expected when compared to direct measurement.

#### *5.6.3.7 Classification of transmitral filling patterns*

Borgarelli and others (1997) were able to classify 10 dogs with DCM and sinus rhythm as either “restrictive” or “non-restrictive”, by combining mitral E/A ratios and deceleration times (Borgarelli *et al.*, 1997). They described dogs with mitral E/A ratios  $>2.0$  as restrictive, and dogs with E/A  $<1.0$  as “non-restrictive”, and classified dogs with mitral E/A ratios between  $1.0 - 2.0$  according to the mitral E deceleration time ( $<0.080$  sec as restrictive,  $>0.080$  sec as non-restrictive).

The classification system used in table 5.15 is similar, although IVRT was also included. The cut-off points used for deceleration time and IVRT are based on the values in the normal GSH pointers. Only one of the normal dogs had a deceleration time less than  $0.070$  sec, and none of the normal dogs had an isovolumic relaxation time less than  $0.036$  when measured from a subcostal aortic recording (although this was the mean IVRT using the left apical aortic velocities). In studies examining prognosis in human patients, deceleration times less than  $0.112 - 0.133$  sec have been associated with a worse prognosis (Giannuzzi *et al.*, 1996; Pozzoli *et al.*, 1995; Shen *et al.*, 1992). Where deceleration time has been used as a predictor of decreased survival, cut-off points have ranged from less than  $0.140$  sec (Xie *et al.*, 1994) to less than  $0.115$  sec (Pinamonti *et al.*, 1993; Pinamonti *et al.*, 1997). These values are all substantially lower than the normal mean value (approximately  $0.180$  sec) reported for people in their third to fourth decade of life (Klein *et al.*, 1994). Mean deceleration time climbs to  $>0.210$  sec for people in their seventh and eighth

decade (Klein *et al.*, 1994). In view of the fact that these human deceleration cut-off points range from 20-40% lower than age-adjusted means, the deceleration time cut-off point used in Borgarelli's study seemed somewhat high, and a shorter deceleration time cut-off point was arbitrarily used in this study. Obviously cut-off points for a classification system would ideally be based on the results of studies examining the association between prognosis and mitral deceleration time in dogs with DCM.

On the basis of the criteria used in this study, adapted from a combination of Borgarelli's criteria, human criteria, and the normal GSH pointer values, the Dobermanns appear to have a predominantly restrictive pattern, and the cockers have a more heterogeneous pattern, showing a greater tendency towards pseudonormal or abnormal relaxation patterns. The improved survival in the cocker spaniels is consistent with their transmitral filling classification, extrapolating from human data. From human transmitral flow patterns, increased survival is most likely with long mitral deceleration times and IVRT, and a reduced mitral E/A ratio (as seen in the cockers). A poor prognosis is expected in human patients with short mitral deceleration times and IVRT, and increased mitral E:A ratios (as seen in the Dobermanns).

#### *5.6.3.8 Mitral regurgitation*

Although there was no significant difference in the grade of mitral regurgitation between the two affected breeds, the median grade was higher in the cockers. Mitral regurgitation was recorded in every affected dog, apart from one Dobermann, which is a higher prevalence than often reported in human DCM

(Blondheim *et al.*, 1991). It is possible that there may have been a higher level of low-grade degenerative mitral valve disease in the cocker spaniels. Although the severity of mitral regurgitation is likely to be worse in primary valvular disease than myocardial disease, severe mitral regurgitation is often reported in human DCM patients, with regurgitant fractions frequently greater than 20%, and sometimes greater than 40% (Blondheim *et al.*, 1991; Keren *et al.*, 1988). Accurate quantification of mitral regurgitation is rarely carried out in dogs. In the past, it has not been practical to perform angiography on a routine basis in clinical canine patients. With current Doppler techniques, including Doppler volumetric techniques and the proximal isovelocity surface area method, there is no practical reason to avoid quantitative estimation of mitral regurgitation in dogs, apart from the lack of reference standards (Brown and Kittleson, 1994).

#### *5.6.3.9 Systolic function assessed by the mitral regurgitation signal*

The velocity of mitral regurgitation relates to the peak systolic pressure gradient developed between the left ventricle and the left atrium. Borgarelli and others (1997) found the peak velocity of mitral regurgitation to be the best Doppler-derived variable predictor of outcome in a small group of dogs with DCM. Dogs with a peak velocity of mitral regurgitation less than 4.0 m/s had only a 23% survival rate at 12 months, compared with 83% survival in dogs with velocities greater than 4.0 m/s. Two cocker spaniels and three Dobermanns had mitral regurgitation velocities less than 4.0 m/s.

The rate of left ventricular pressure development (LV dP/dt) derived from the mitral regurgitation spectral signal was significantly lower in the cocker spaniels,



suggesting worse systolic function. Although LV  $dP/dt$  is independent of aortic pressure if measured during isovolumic contraction, it may be influenced by preload (Van Den Bos *et al.*, 1973). It is generally regarded as adequate for assessing directional changes in contractility, but less suitable for comparisons between individuals. If both groups of dogs had equivalent systolic dysfunction, but the cockers had lower left atrial pressures and therefore lower preload, it is possible that this might have accounted for the difference in  $dP/dt$ .

#### 5.6.3.10 *Tricuspid regurgitation*

Tricuspid regurgitation appeared to be less prevalent than mitral regurgitation. The main value of tricuspid regurgitation is in the calculation of right ventricular systolic pressure, which permits the recognition of pulmonary hypertension. Out of three Dobermanns in which it was possible to measure the velocity of tricuspid regurgitation, one had a peak velocity of tricuspid regurgitation of 3.3 m/s (estimated pulmonary artery systolic pressure of 44 mmHg). Two out of four cockers with measurable tricuspid regurgitation signals had a peak tricuspid regurgitation velocity of greater than 2.5 m/s. Pulmonary hypertension has been defined as pulmonary artery systolic pressures greater than 35mmHg, or tricuspid regurgitation velocities greater than 2.5 m/s (equivalent to systolic pulmonary artery pressures of approximately 35mmHg, once right atrial pressures are added to the trans-tricuspid gradient) (Oh *et al.*, 1999d). Tricuspid regurgitation velocities  $>2.5$ m/s have been associated with a poor prognosis in human DCM patients (Abramson *et al.*, 1992; Grzybowski *et al.*, 1996).

#### 5.6.4 Further Doppler applications

Recent reports have suggested a number of Doppler-derived variables that may prove useful in the assessment of canine DCM. Pulmonary venous flow velocities may provide information on left ventricular end-diastolic pressure. The atrial reversal wave duration compared with mitral A wave duration has been shown to be a useful predictor of left ventricular end-diastolic pressures (Ito *et al.*, 1998; Nakatani *et al.*, 1994; Rossvoll and Hatle, 1993). Values for normal pulmonary venous atrial reversal flow duration in dogs have been published (Schober *et al.*, 1998), but further attention is warranted in dogs with DCM.

The recently reported “index of myocardial performance” should be easy to apply to canine Doppler recordings, and once normal values are established, should offer a simple means of assessing global left and right ventricular performance (Dujardin *et al.*, 1998; Tei *et al.*, 1996; Tei *et al.*, 1995). The colour M-mode derived velocity of left ventricular flow propagation should also be applicable to dogs with DCM, and should offer some means of distinguishing normal mitral inflow from “pseudonormal” (Garcia *et al.*, 1997; Garcia *et al.*, 1999; Takatsuji *et al.*, 1996). Pulsed wave Doppler tissue imaging is another means of differentiating between normal and pseudonormal mitral inflow (Sohn *et al.*, 1997).

#### 5.7 CONCLUSIONS

Transvalvular velocities can be recorded with DE in conscious, unsedated normal dogs and dogs with dilated cardiomyopathy. The reproducibility of peak velocities is adequate, although acceleration and deceleration times and isovolumic relaxation times are less repeatable. Aortic velocities are easier to record from the

left apex than the subcostal view, and isovolumic relaxation time should be measured directly from a spectral Doppler recording with the sample volume placed between the mitral inflow tract and the aortic outflow tract.

Marked differences were found in most of the recorded Doppler-derived variables between the normal dogs and the dogs with DCM, indicating abnormalities in systolic and diastolic performance in the affected dogs. For the most part, there were no differences between the affected Dobermanns and cocker spaniels, with the notable exception of key transmitral flow variables, and LV  $dP/dt$  derived from the mitral regurgitation signal. The mitral valve E/A ratio was significantly higher in the Dobermanns than the cockers, a finding that has been associated with a worse prognosis in human DCM patients. A shortened isovolumic relaxation time has also been associated with raised filling pressures and a poorer prognosis, and the Dobermanns, once again, had significantly shortened isovolumic relaxation times compared with the cockers.

From this study, it appears that assessment of left ventricular diastolic function and left heart filling pressures may provide useful information in the prediction of outcome in dogs with DCM, and such indices would have the additional merit of being applicable across breeds. Further investigation of canine DCM with Doppler echocardiography is certainly warranted, on the basis of the results presented here.

## CHAPTER 6: INTEGRATED BACKSCATTER MEASUREMENT IN NORMAL DOGS AND DOGS WITH DCM

### 6.1 INTRODUCTION

The manner in which biological tissue interacts with high frequency ultrasound waves can be exploited to provide non-invasive yet quantitative information about myocardial tissue characteristics. Ultrasonic integrated backscatter (or the cyclic variation in integrated backscatter) has been applied to myocardial disease in animal models and to human cardiac disease, providing information on myocardial composition and function. Ultrasonic tissue characterisation is a non-invasive technique that yields unique information, but availability has hitherto limited access in a clinical setting.

To the author's knowledge, there have been no reports of ultrasonic IB measurement in dogs with naturally occurring cardiac disease.

### 6.2 AIMS

1. To assess the feasibility and repeatability of measuring the cyclic variation of ultrasonic integrated backscatter in unsedated conscious dogs, using a protocol previously validated in open chest pigs (Moran *et al.*, 1994).

2. To determine if the cyclic variation in integrated backscatter is reduced in dogs with abnormal myocardial function (Dobermanns and cocker spaniels with dilated cardiomyopathy) compared with normal dogs (healthy German shorthaired pointers with no evidence of myocardial dysfunction).

## 6.3 BACKGROUND

### 6.3.1 IB studies in human DCM

The magnitude of cyclic variation in integrated backscatter (CVIB) has been related to myocardial contractile function in a number of animal studies, and as early as 1987, Vered and others applied the technique to five human subjects with dilated cardiomyopathy (DCM) and compared CVIB with controls (Vered *et al.*, 1987). The DCM patients had either absent or reduced magnitude of CVIB.

Since this report, there have been several studies of CVIB in both adult DCM (Bouki *et al.*, 1996; Fujimoto *et al.*, 1999; Naito *et al.*, 1996a; Naito *et al.*, 1996b; Zuber *et al.*, 1999) and in DCM in children (Baysal *et al.*, 1991; Goens *et al.*, 1996). All have found reduced mean CVIB in DCM groups compared with controls, although some studies have identified individuals with DCM with normal CVIB in some regions (Bouki *et al.*, 1996; Naito *et al.*, 1996a). In the study by Naito and co-workers (1996), dobutamine was used as a stress test to unmask abnormal CVIB. Ten DCM patients with normal CVIB at rest showed a similar increase in wall thickening to control subjects, but failed to demonstrate an increase in CVIB with dobutamine, as seen in the controls. In one of the studies of children with DCM, four patients showed an improvement in systolic function over time, but their CVIB values remained depressed (Goens *et al.*, 1996).

Few studies have examined absolute IB levels in human DCM, reflecting the difficulty in calculating absolute IB levels *in vivo*. Picano's group reported IB levels as a percentage of the pericardial backscatter signal in a group of DCM patients who underwent left ventricular (LV) myocardial biopsy sampling (Picano *et al.*, 1990). They found that IB levels increased as the percentage of connective tissue in LV myocardial samples increased, with patients having connective tissue percentages >20% showing significantly higher IB levels. Baysal and others (1991) reported "integrated backscatter" levels in 14 children with DCM, but no attempt was made to calibrate the system (a Toshiba Sonolayer SSA-90A) or to calibrate for inter-subject factors, and histogram values were reported rather than decibels, so these findings should be viewed with caution (Baysal *et al.*, 1991). Naito and co-workers (1996) also reported IB levels in DCM patients with respect to LV biopsy results (Naito *et al.*, 1996b). They found a correlation between IB levels and the volume fraction of interstitial fibrosis (with a correlation coefficient of 0.68), but no correlation between volume fraction of fibrosis and CVIB. Another study comparing CVIB in adult DCM patients with percentage fibrosis area found reduced CVIB compared with controls, but a better correlation between CVIB and mean myocyte diameter than with percentage fibrosis area (Fujimoto *et al.*, 1999).

Angermann's group (1986) also reported patients with dilated cardiomyopathy to have reduced cyclic variation in myocardial texture characteristics, such as grey level co-occurrence matrices. Texture analysis techniques often use videodensitometry to measure mean grey scale levels, but this study actually employed radiofrequency signals.

## 6.4 MATERIALS AND METHODS

### 6.4.1 Animals

#### *6.4.1.1 Normal dogs*

Thirteen normal German shorthaired (GSH) pointers were studied. All dogs were considered to be healthy on the basis of historical findings and physical examination. All dogs were examined by two-dimensional, M-mode and Doppler echocardiography to rule out obvious cardiac disease.

#### *6.4.1.2 Dogs with DCM*

A total of 13 dogs with DCM were studied. Every affected dog had a history of congestive heart failure, but all were stable on therapy at the time of scanning. The diagnosis of DCM was based on echocardiographic evidence of left ventricular dilation in the absence of a congenital cardiac or valvular morphological abnormality, and evidence of reduced contractile function (LV fractional shortening < 20%). Nine of the DCM dogs were Dobermanns (Dobermanns 3, 4, 6, 7, 8, 9, and 10 described in chapter 2, plus two other affected Dobermanns), and the other four were English cocker spaniels (Cockers 2, 4, 5 and 6 described in chapter 2). Of the Dobermanns not previously described, one was a 12 year old male of 38 kg with atrial fibrillation, and the other was an 8 year old neutered female with sinus rhythm.

### 6.4.2 Imaging system and RF data acquisition

A modified commercially available digital echocardiography system (ATL Ultramark 9, Advanced Technology Laboratories, Bothell, Washington, USA) with a 5 MHz phased array transducer (bandwidth 2.5 - 7.5 MHz) and simultaneous ECG

was used to acquire the two-dimensional echocardiographic images (Fig 6.1). Dogs were positioned in right lateral recumbency on a modified table, to allow placement of the transducer on the thoracic wall from below. Right parasternal long axis views were used to obtain images of the left ventricle, as this view has resulted in the most consistent cyclic variation in integrated backscatter (Lange *et al.*, 1995; Vandenberg *et al.*, 1989). Transmit power and time gain compensation (TGC) controls were set at pre-adjusted levels for the studies, and the gain was adjusted at the start of each study, then was kept constant. The RF signals were digitised at 12 MHz to 16 bit.

Fifteen consecutive frames of RF data were collected, and manually controlled so that the first frame occurred shortly before end-diastole. Each echo line of RF data consisted of a maximum of 4,096 samples, depending on the image depth. A single image consisted of 128 lines with up to 4,096 samples/line at 2 bytes/sample, occupying 1 MB of storage memory/ frame. The RF acquisition system was equipped with a 16 MB memory board, of which 1 MB was reserved for control registers so that a maximum of 15 complete frames of RF data could be acquired. The two-dimensional images were also stored on video, and hard copies were made of each frame.

The RF data were collected from each scan and downloaded onto a workstation (Sparc System LX, Sun Microsystems Inc., Mountain View, California, USA) for signal processing. The RF data were rectified, low-pass filtered, logarithmically-compressed and scan-converted before the reconstruction of two-dimensional images similar to the images obtained by the original echocardiography machine. Myocardial regions of interest in the reconstructed images were chosen within the left ventricular free wall and interventricular septum, using a mouse-



driven cursor (Figure 6.2). Care was taken to avoid the specular echoes of the endocardial and epicardial surfaces. Regions of interest in the same myocardial area were tracked through each frame. These data were then related to the original uncompressed RF data, and the IB value was calculated. A set of calibration scans was also recorded, using a standard grey-scale test tissue phantom (Diagnostic Sonar, Livingston, UK) to compensate for the effects of transducer characteristics, TGC, depth, and dynamic range settings of the ultrasound machine (Figure 6.3). The calibration scans were recorded for each depth and focus setting used during the *in vivo* scans, and the RF data were downloaded to the workstation in similar manner. The regions of interest chosen for each canine scan were then superimposed on the phantom scan RF data, and the IB was calculated according to the following formula:

Formula 6.1:

$$IB = \frac{\int_{t-\Delta t}^{t+\Delta t} |V(t)|^2 \delta t}{\int_{t-\Delta t}^{t+\Delta t} |P(t)|^2 \delta t}$$

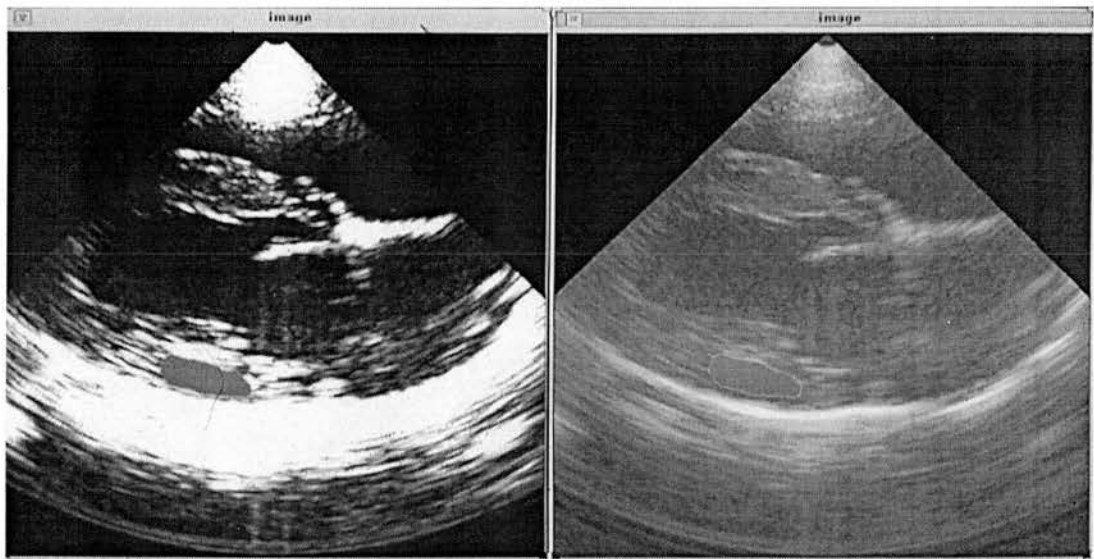
where  $V(t)$  is the amplitude of the uncompressed RF signal from the myocardial region of interest,  $P(t)$  is the amplitude of the uncompressed RF signal from the phantom,  $\delta t$  is a small increment of time over the timegate, and  $2\Delta t$  is the timegate over the region of interest. The value was then converted to the decibel (dB) notation by taking the logarithm and multiplying by 10.

Figure 6.1: Ultrasound system used for RF data acquisition



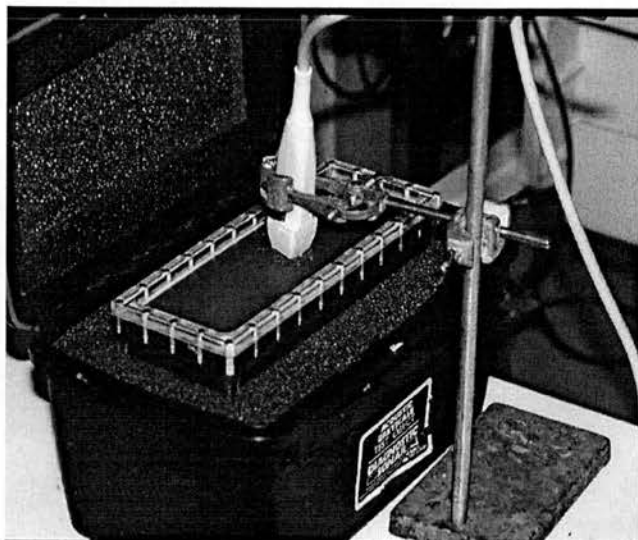
Modified ATL Ultramark 9 echocardiography system used for the integrated backscatter studies (Advanced Technology Laboratories, Bothell, Washington, USA).

Figure 6.2: Selection of region of interest within the left ventricular free wall.



Original digital image acquired by ATL Ultramark 9 (left), and reconstructed image following rectification, low-pass filtering, log compression and scan conversion (right). A region of interest is shown in the basal left ventricular free wall, avoiding the endocardial and epicardial interfaces, and the papillary muscles.

Figure 6.3: Grey scale tissue test phantom.



Grey scale tissue test phantom (Diagnostic Sonar, Livingston, UK), showing placement of the transducer for acquisition of the calibration scans at the same TGC, depth and range settings of the ATL Ultramark 9 used for the cardiac scans.

### 6.4.3 Protocol

#### *6.4.3.1 Normal dogs*

Thirty-one scans were obtained in total in the normal GSH pointers (three dogs were scanned four times, one dog was scanned three times, seven dogs were scanned twice, and two dogs were scanned once). For 26 scans, values for IB were obtained for three myocardial regions (PW1: the basal left ventricular free wall, PW2: the apical left ventricular free wall, and SP: the interventricular septum). For five scans, values were obtained in two myocardial regions (the basal left ventricular free wall, and the interventricular septum). Each myocardial region was obtained over 15 frames. In this manner, a set of IB values with 15 frames for each cardiac cycle was acquired in 88 sites.

#### *6.4.3.2 Dogs with DCM*

Twenty scans were obtained in total in the dogs with DCM. Thirteen scans were obtained in the Dobermanns. Four dogs were scanned twice each, and the other five dogs were scanned once each. Seven scans were obtained in the cocker spaniels, with one dog being scanned three times, one dog being scanned twice, and two dogs being scanned once each. For all 20 scans, values were obtained for IB in two regions: the basal left ventricular free wall (PW1), and the interventricular septum (SP). As with the GSPs, values for each region were obtained for a total of 15 frames over one cardiac cycle.

#### 6.4.4 Analysis of data

The mean value was subtracted from the value for each frame, to yield the variation about the mean (designated as 0 dB). Using the concurrently recorded ECG, the 15 frames of RF data were synchronised for each scan by defining the end-diastolic frame as the frame recorded at the start of the R wave, and the end-systolic frame as the frame with the smallest left ventricular volume. Cyclic variation was calculated in two ways: (a) as the difference between the end-diastolic value and the end-systolic value ( $CV_{ED-ES}$ ), and (b) as the difference between the maximal and minimal values ( $CV_{max-min}$ ).

##### *6.4.4.1 Statistical analysis*

All values are expressed as mean  $\pm$  SD unless otherwise stated. Repeatability was assessed in eleven normal GSH pointers by comparing two scans recorded within 30 minutes of each other. End-diastolic values, end-systolic values and amplitude of cyclic variation ( $CV_{ED-ES}$  and  $CV_{max-min}$ ) were compared between the first and second scan in each region using a paired t-test (when values were normally distributed and of equal variance). When values failed a test for normal distribution and equal variances, a Mann-Whitney rank sum test was performed.

Values for  $CV_{ED-ES}$  and  $CV_{max-min}$  in the septum and free wall were compared in the normal dogs and the two groups of dogs with DCM using a one-way analysis of variance (where data were normally distributed with equal variances), or using a Kruskal-Wallis one way analysis of variance on ranks (where tests for normality and equal variances were failed). When significant differences were found between the three groups at a significance level of  $p < 0.05$ , multiple pairwise

comparisons were made using Dunn's method. In each group of dogs (GSH pointers, Dobermanns and cocker spaniels), values for  $CV_{ED-ES}$  were compared in each region of interest using either a t-test, or a Mann-Whitney rank sum when data were not normally distributed or had differing variances. Within-group values for  $CV_{max-min}$  were also compared for each region.  $CV_{ED-ES}$  values were compared with  $CV_{max-min}$  values for each region within the same group. All statistical analyses were carried out using a proprietary statistical software programme (SigmaStat 2.0, Jandel Scientific, San Rafael, CA, USA). Graphs were plotted using SigmaPlot (Jandel Scientific, San Rafael, CA, USA).

## 6.5 RESULTS

### 6.5.1 Normal dogs

Of the 13 normal German shorthaired pointers, 8 were female and 5 were male. Mean age was 6.0 (range, 2 to 12) years, and mean bodyweight ( $\pm$  SD) was 26.9 ( $\pm$  3.8) kg. All dogs were in sinus rhythm or sinus arrhythmia, and mean heart rate was  $83.3 \pm 15.8$  bpm.

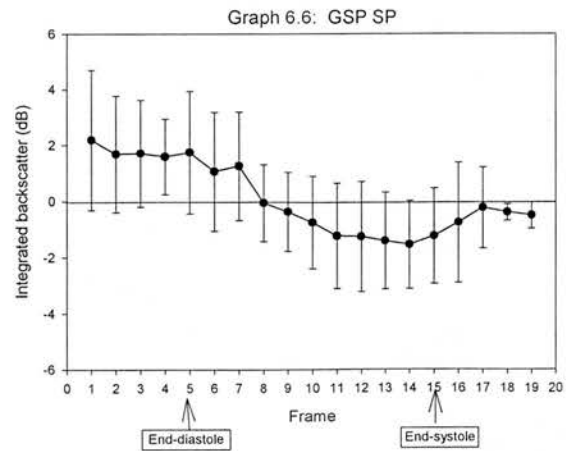
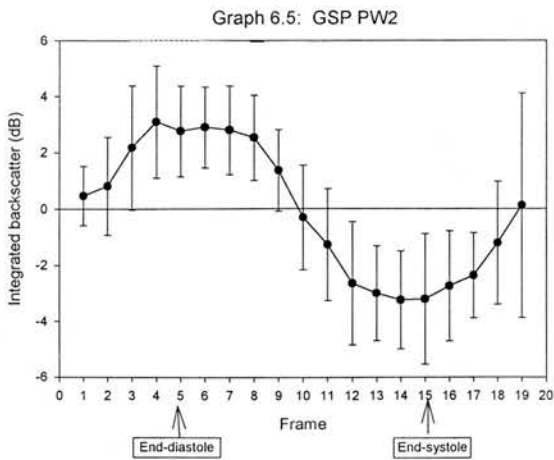
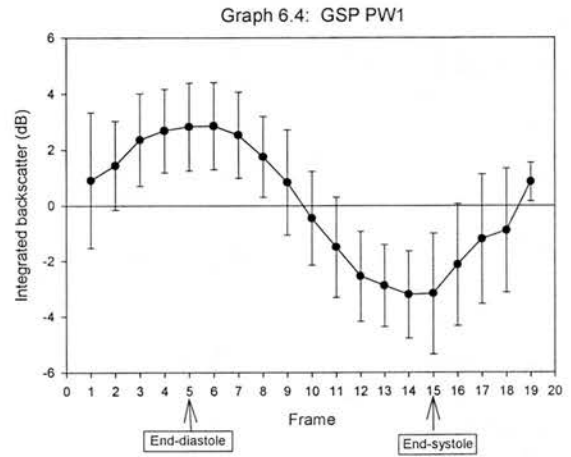
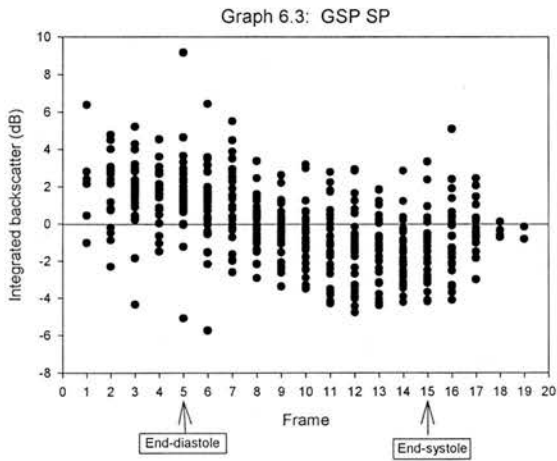
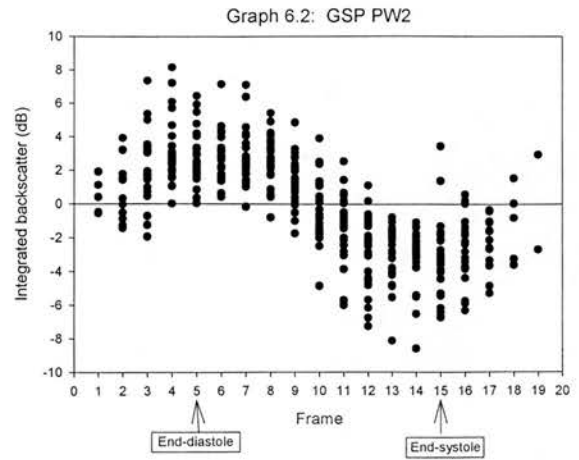
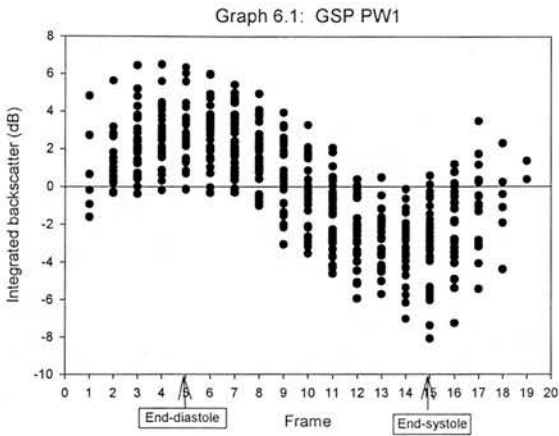
Table 6.1 Values for integrated backscatter in normal GSPs on two consecutive scans

IB (dB)	Scan	PW1		PW2		SP	
End-diastole	Scan 1	3.31 ± 1.18	NS	2.81 ± 1.83	NS	2.63 ± 2.51	NS
	Scan 2	3.31 ± 1.52		2.57 ± 2.00		0.62 ± 2.22	
End-systole	Scan 1	-3.62 ± 2.28	NS	-2.84 ± 2.57	NS	-1.53 ± 1.73	NS
	Scan 2	-3.49 ± 2.40		-3.38 ± 2.49		-0.68 ± 1.82	
CV <sub>ED-ES</sub>	Scan 1	6.93 ± 2.92	NS	5.65 ± 3.80	NS	4.17 ± 3.30	NS
	Scan 2	6.80 ± 3.35		5.95 ± 3.48		1.30 ± 3.60	
CV <sub>max-min</sub>	Scan 1	8.97 ± 2.04	NS	9.02 ± 2.22	NS	7.23 ± 3.00	NS
	Scan 2	9.35 ± 2.49		9.75 ± 3.74		4.97 ± 5.28	

GSPs: German short-haired pointers, IB: integrated backscatter, dB: decibels, PW1: basal left ventricular free wall, PW2: apical left ventricular free wall, SP: interventricular septum, CV<sub>ED-ES</sub>: cyclic variation of integrated backscatter (end-diastolic value – end-systolic value), CV<sub>max-min</sub>: maximum amplitude in cyclic variation of integrated backscatter throughout cardiac cycle, NS: not significantly different at the p<0.05 level

The results of scans on two consecutive days in 11 normal GSPs are shown in table 6.1, listing end-diastolic and end-systolic values, and values for CV<sub>ED-ES</sub> and CV<sub>max-min</sub>. Although no significant differences were found on successive scans for any of the regions, values appeared more reproducible for the left ventricular free wall than the interventricular septum. Graphs 6.1- 6.3 show plots of the raw data values for IB over 15 frames in the normal GSPs for PW1, PW2 and SP. Graphs 6.4 - 6.6 show the mean values ± SD for all scans in the normal dogs in the three regions of interest. Table 6.2 shows mean values for CV<sub>ED-ES</sub> and CV<sub>max-min</sub> for the two regions within the LV free wall and the interventricular septum over the 15 frames. Table 6.3 shows the results of statistical comparisons within the regions, and between the two methods of calculating cyclic variation.

Integrated backscatter in normal GSH pointers





The mean  $CV_{ED-ES}$  was greatest ( $p < 0.05$ ) in the basal portion of the left ventricular free wall (PW1), with a mean  $\pm$  SD value of  $6.00 \pm 3.17$  dB. The apical portion of the left ventricular free wall was similar ( $5.97 \pm 3.25$  dB), but the interventricular septum showed significantly less ( $p < 0.05$ ) cyclic variation ( $2.97 \pm 3.29$  dB). The  $CV_{max-min}$  was significantly higher than for  $CV_{ED-ES}$  in all regions ( $p < 0.003$  for the PW1 region,  $p < 0.001$  for PW2 and SP).  $CV_{max-min}$  was greatest ( $p < 0.05$ ) in the PW2 region of the free wall ( $8.95 \pm 2.91$  dB), with a similar value in the PW1 region ( $8.12 \pm 2.63$  dB) but a significantly lower value ( $p < 0.05$ ) in the septum ( $6.42 \pm 2.45$  dB).

Table 6.2: Values for cyclic variation in integrated backscatter

Group	Cyclic Variation method	PW1 (dB)	PW2 (dB)	SP (dB)
<b>GSPs</b>	$CV_{ED-ES}$	$6.00 \pm 3.17$	$5.97 \pm 3.25$	$2.97 \pm 3.29$
	$CV_{max-min}$	$8.12 \pm 2.63$	$8.95 \pm 2.91$	$6.42 \pm 2.45$
<b>Dobermanns</b>	$CV_{ED-ES}$	$-0.01 \pm 3.25$	-	$-2.63 \pm 6.67$
	$CV_{max-min}$	$6.58 \pm 1.91$	-	$7.57 \pm 6.48$
<b>Cockers</b>	$CV_{ED-ES}$	$-0.33 \pm 4.01$	-	$0.01 \pm 1.95$
	$CV_{max-min}$	$7.45 \pm 3.20$	-	$7.33 \pm 3.65$

GSPs: German short-haired pointers, dB: decibels, PW1: basal left ventricular free wall, PW2: apical left ventricular free wall, SP: interventricular septum,  $CV_{ED-ES}$ : cyclic variation of integrated backscatter (end-diastolic value – end-systolic value),  $CV_{max-min}$ : maximum amplitude in cyclic variation of integrated backscatter throughout cardiac cycle

### 6.5.2 Dogs with DCM

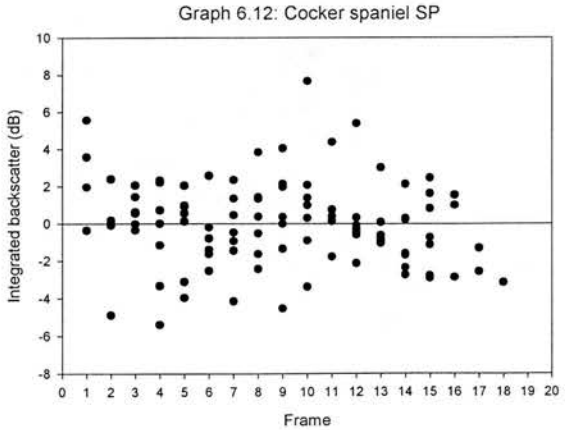
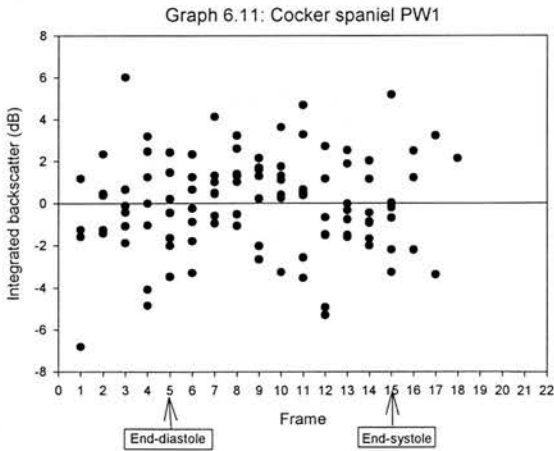
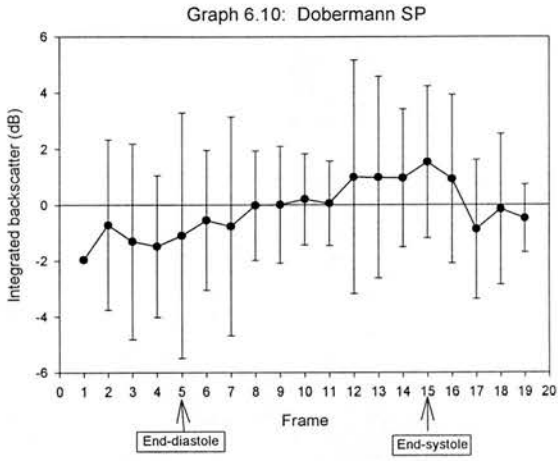
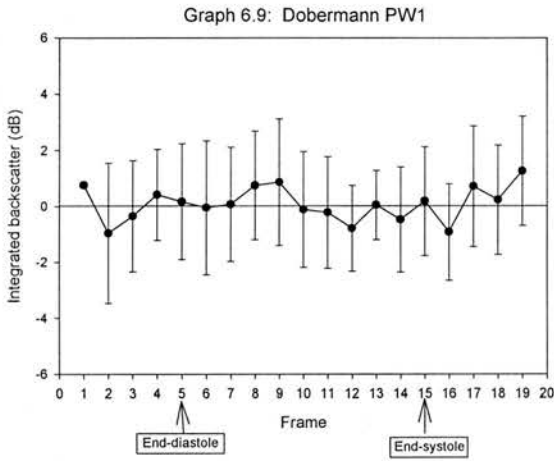
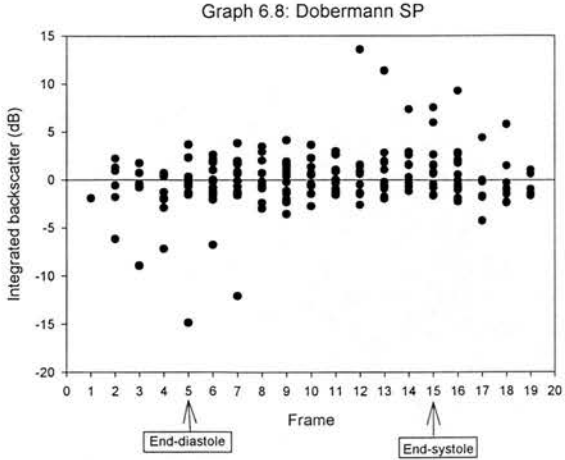
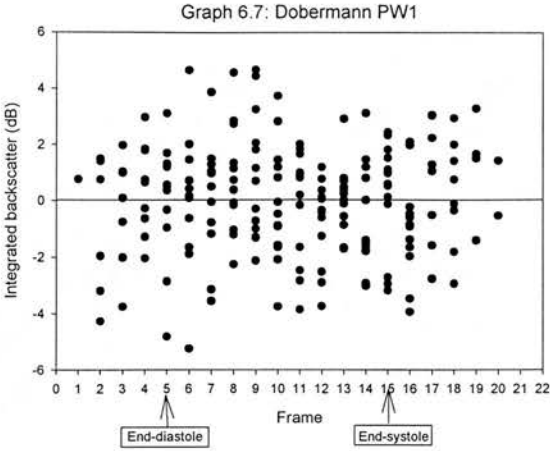
Mean bodyweight for the DCM group as a whole was  $31.5 \pm 12.3$  kg, mean age was  $7.8 \pm 2.1$  years, and mean heart rate was  $145.3 \pm 28.5$  bpm. Nine of the

DCM dogs were Dobermanns (2 females, 7 males), with a mean age for this group of 9.1 (range, 6 to 12) years, and a mean bodyweight of  $37.9 \pm 8.0$  kg. The rhythm in three of the Dobermanns was atrial fibrillation, but was sinus rhythm in the other six. The other four dogs with DCM were cocker spaniels (all males). Mean age was 6.5 years (range 3 to 10 years), and mean bodyweight was  $14.9 \pm 2.0$  kg. All the cocker spaniels were in sinus rhythm.

The raw data points for the LV free wall (PW1) and interventricular septum (SP) in the Dobermanns with DCM are displayed in graphs 6.7 – 6.8, with mean  $\pm$  SD values plotted in graphs 6.9 and 6.10. Graphs 6.11 and 6.12 show the raw data points for PW1 and SP in the cocker spaniels with DCM, with mean  $\pm$  SD values plotted in graphs 6.13 and 6.14. Cyclic variation in IB is shown for the two regions in each group in table 6.2.

There were marked differences in the amplitude of cyclic variation according to the method of calculation, with a mean PW1 value in the Dobermanns for  $CV_{ED-ES}$  of  $-0.01 \pm 3.25$  dB, compared with a value for  $CV_{max-min}$  of  $6.58 \pm 1.91$  dB ( $p < 0.001$ ). Findings were similar in the cocker spaniels, with a  $CV_{ED-ES}$  of  $-0.33 \pm 4.01$  dB compared with a  $CV_{max-min}$  of  $7.45 \pm 3.2$  dB ( $p < 0.02$ ). A similar difference was seen between the two methods in the septum, with a mean  $CV_{ED-ES}$  lower than the mean  $CV_{max-min}$  in both DCM breeds. For the Dobermanns,  $CV_{ED-ES}$  was  $-2.63 \pm 6.67$  dB versus  $7.57 \pm 6.48$  dB for  $CV_{max-min}$  ( $p < 0.001$ ), and for the cockers,  $CV_{ED-ES}$  was  $0.01 \pm 1.95$  dB versus  $7.33 \pm 3.65$  dB for  $CV_{max-min}$  ( $p < 0.007$ ). In contrast with the normal dogs, in both DCM groups the values for both  $CV_{max-min}$  and  $CV_{ED-ES}$  were similar in the free wall and the septum.

Integrated backscatter in Dogs with DCM



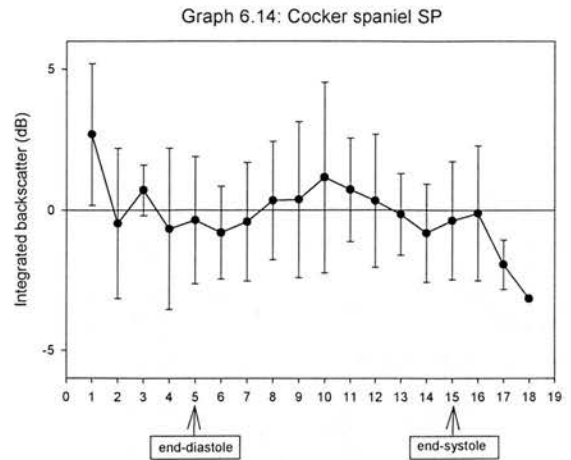
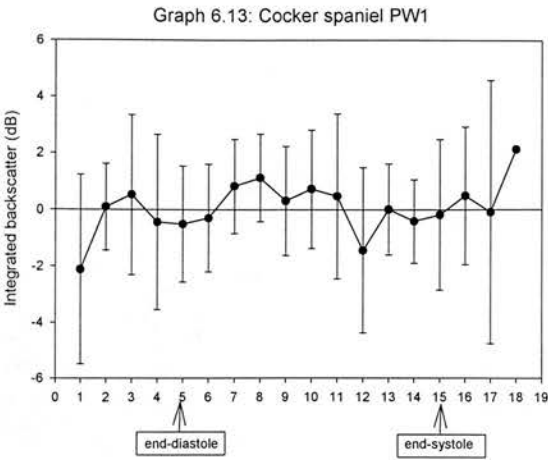


Table 6.3: Comparison of groups, regions, and cyclic variation measurements

<b>GSPs</b>	CV <sub>ED-ES</sub>	PW1 <sup>a</sup> vs PW2 <sup>a</sup> vs SP <sup>b</sup>	p < 0.001
	CV <sub>max-min</sub>	PW1 <sup>a</sup> vs PW2 <sup>a</sup> vs SP <sup>b</sup>	p < 0.002
	PW1	CV <sub>ED-ES</sub> vs CV <sub>max-min</sub>	p < 0.003
	PW2	CV <sub>ED-ES</sub> vs CV <sub>max-min</sub>	p < 0.001
	SP	CV <sub>ED-ES</sub> vs CV <sub>max-min</sub>	p < 0.001
<b>Dobermanns</b>	CV <sub>ED-ES</sub>	PW1 vs SP	NS
	CV <sub>max-min</sub>	PW1 vs SP	NS
	PW1	CV <sub>ED-ES</sub> vs CV <sub>max-min</sub>	p < 0.001
	SP	CV <sub>ED-ES</sub> vs CV <sub>max-min</sub>	p < 0.001
<b>Cockers</b>	CV <sub>ED-ES</sub>	PW1 vs SP	NS
	CV <sub>max-min</sub>	PW1 vs SP	NS
	PW1	CV <sub>ED-ES</sub> vs CV <sub>max-min</sub>	p < 0.02
	SP	CV <sub>ED-ES</sub> vs CV <sub>max-min</sub>	p < 0.007
<b>CV<sub>ED-ES</sub></b>	PW1	GSP <sup>a</sup> vs Dob <sup>b</sup> vs CSp <sup>b</sup>	p < 0.001
	SP	GSP <sup>a</sup> vs Dob <sup>b</sup> vs CSp <sup>b</sup>	p < 0.001
<b>CV<sub>max-min</sub></b>	PW1	GSP vs Dob vs CSp	NS
	SP	GSP vs Dob vs CSp	NS

Groups with the same superscript were not significantly different at the P<0.05 level in pairwise comparisons.

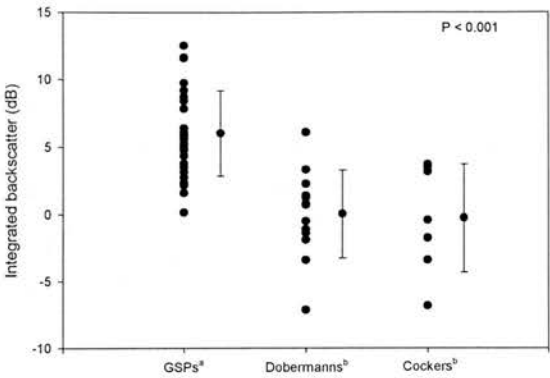
GSPs: German short-haired pointers, IB: integrated backscatter, dB: decibels, PW1: basal left ventricular free wall, PW2: apical left ventricular free wall, SP: interventricular septum, CV<sub>ED-ES</sub>: cyclic variation of integrated backscatter (end-diastolic value – end-systolic value), CV<sub>max-min</sub>: maximum amplitude in cyclic variation of integrated backscatter throughout cardiac cycle, NS: not significantly different at the p<0.05 level

### 6.5.3 Comparison between normal dogs and dogs with DCM

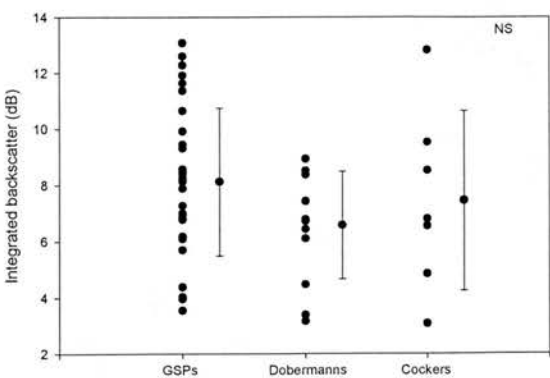
The amplitude of cyclic variation in the PW1 region in the three groups is displayed in graph 6.15 ( $CV_{ED-ES}$ ) and graph 6.16 ( $CV_{max-min}$ ). Septal amplitude of cyclic variation is shown in graph 6.17 ( $CV_{ED-ES}$ ) and graph 6.18 ( $CV_{max-min}$ ). Significant differences were found between the three groups in both regions using the  $CV_{ED-ES}$  method ( $p < 0.001$ ), with the two DCM groups having lower values for cyclic variation ( $p < 0.05$ ) compared with the normal dogs, but similar values to each other. No significant differences were found between the normal dogs and the dogs with DCM in either region when the  $CV_{maxamp}$  method was used.

Comparison of cyclic variation in integrated backscatter in normal German short-haired pointers, and in Dobermanns and cocker spaniels with DCM.

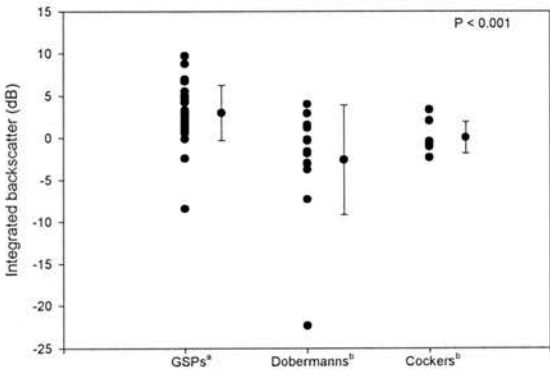
Graph 6.15: PW1 cyclic variation in IB (ED-ES)



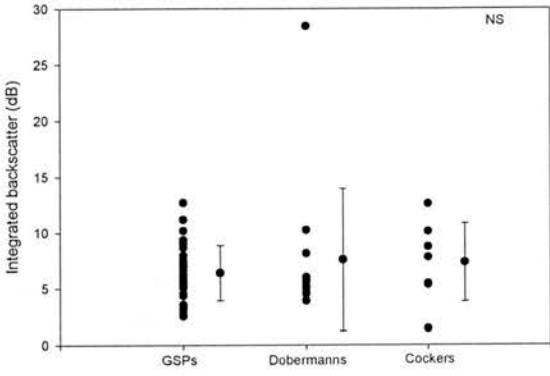
Graph 6.16: PW1 cyclic variation in IB (max-min)



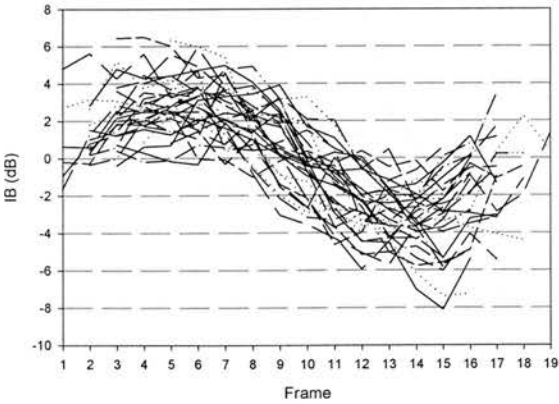
Graph 6.17: SP cyclic variation in IB (ED-ES)



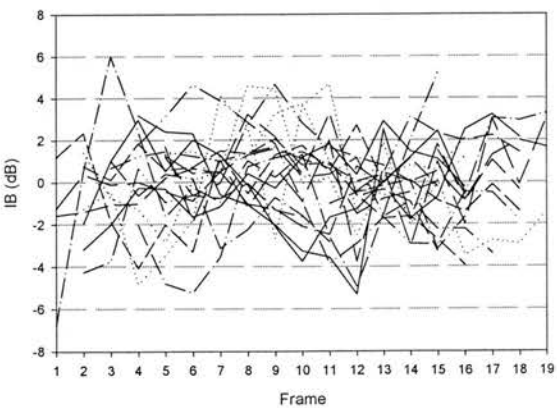
Graph 6.18: SP cyclic variation in IB (max-min)



Graph 6.19: PW1 scans in individual GSH pointers



Graph 6.20: PW1 in individual dogs with DCM



## 6.6 DISCUSSION

### 6.6.1 Study protocol

Measurement of integrated backscatter *in vivo* is fraught with difficulties when attempting to assess absolute levels of integrated backscatter, for reasons that include the effects of the intervening tissue of the chest wall, respiratory variation in lung tissue between the transducer and the myocardium, and anisotropy. *In vitro* assessment allows calibration using a perfect reflector, but calibration *in vivo* is more difficult. In this study, backscatter measurements were calibrated using a tissue phantom, as described by Yao and co-workers (Yao *et al.*, 1990). Although this was not sufficient to allow measurement of absolute backscatter levels, using the ratio of the signal from the canine scans and the phantom scans helped to eliminate the effects of the transducer characteristics. Although attenuation of the RF signal by the thoracic wall will inevitably vary between systole and diastole in closed chest dogs, the IB values reported in this study correspond with those previously reported in open chest dogs, suggesting that these effects were probably minimal (Barzilai *et al.*, 1990; Mohr *et al.*, 1989; Naito *et al.*, 1995).

A potential problem associated with the described protocol is the use of a relatively high frequency 5 MHz probe, although digitisation of the RF signal was carried out at 12 MHz, thus introducing the possibility of signal aliasing. However, a 4<sup>th</sup> order Butterworth filter was used within the echocardiography machine to limit aliasing and reduce noise. Calculations derived from this system suggest that <12% of the signal power was likely to be derived from the aliased signal.

Another limitation of this protocol was the inability to acquire more than 15 frames/scan. As with nearly any other cardiac variable, some beat-to-beat variation is inevitable, and this study assessed only one cardiac cycle per scan. An alternative approach would have been to trigger RF data acquisition using the concurrent ECG, so that five frames would be recorded for each cardiac cycle, providing information on up to three cardiac cycles per scan. This protocol was used initially, but led to problems with the DCM dogs, where fewer frames per cardiac cycle made it difficult to predict the true maximum and minimum IB values, because of the effects of phase shifts. Subsequent scans (and all the scans reported here) used continuous frame acquisition. Lack of an integrated timing reference meant that timing of individual data points had to be derived from hard copy printouts of the conventional image. The simultaneous ECG was linked to the frames of the conventional scan, and not to the RF data specifically.

The advantage of the described protocol is the ability to collect RF data from the entire two-dimensional scan image. This provides freedom for the observer to choose specific regions of interest during subsequent off-line analysis, instead of limiting the data acquisition to regions predetermined at the time of scanning.

## 6.6.2 Normal dogs

### *6.6.2.1 Reproducibility*

The cyclic nature of integrated backscatter makes the assessment of sources of variation particularly difficult. In the part of this study that assessed reproducibility, 11 dogs were scanned twice consecutively by one operator, and



measured off-line by the same operator. Under these settings, no significant differences were found over the two scans for end-diastolic and end-systolic values of integrated backscatter, or for the amplitude of cyclic variation in integrated backscatter (whether measured as the difference between end-diastolic and end-systolic values, or as the maximum amplitude over the cardiac cycle). Nevertheless, the statistical power in each of these calculations was suboptimal, and it is possible that increasing the sample size might have resulted in statistically significant differences. Potential contributors to sources of variability include beat-to-beat variation in contractile function (especially under conditions of sinus arrhythmia), variation in scanning planes (leading to differences in anisotropy) and measurement variation. The latter two factors should have been minimised by using the same observer for both scans, and diurnal variation was minimised by recording the scans within less than 30 minutes of each other.

Bijnens and others (1999) examined variability in IB using tissue-mimicking phantoms, and reported standard deviations up to  $\pm 20\%$  (Bijnens *et al.*, 1999). Such large variations in the absence of any variation in the interrogated tissue was believed to be related to signal interference and phase cancellation. Phase cancellation may have less influence on the magnitude of cyclic variation in IB compared with absolute IB levels, which may account for the better reproducibility in this study.

A more detailed study of the individual sources of variance in this group of dogs has been reported, which included assessment of intradog, interdog and interobserver variability (Luis Fuentes *et al.*, 1997). In this report, the scans used in

the present study were included amongst other scans, and three observers measured regions of interest in the PW1, PW2 and SP regions. When all regions of interest in all scans were combined, the intradog variance ranged from  $0.94 \text{ dB}^2$  for end-diastole to  $1.08 \text{ dB}^2$  for end-systole, decreasing to approximately zero at mid-systole. Inter-dog variance was lowest at end-diastole ( $0.45 \text{ dB}^2$ ) and end-systole ( $0.78 \text{ dB}^2$ ), and highest at mid-systole ( $1.75 \text{ dB}^2$ ). Variance components attributable to different observers (whether within each dog or within each scan) were smaller, reaching a maximum of  $0.54 \text{ dB}^2$  for observer variance within each scan at end-systole. These values appear satisfactory, although the variance components were not analysed separately according to region of interest in this report.

Inspection of the values in the present study suggests that at least for specified points within the cardiac cycle, results of IB are adequately reproducible for the left ventricular free wall, but vary more for the interventricular septum. The same appears to be true for the amplitude of cyclic variation. This concurs with good reproducibility seen in a study by Loomis and others (1990) using a different analysis (Loomis *et al.*, 1990), where correlation coefficients of 0.93 and 0.72 were seen for intra- and inter-observer variability respectively in measuring the same scans 6 weeks apart.

Reasons for the greater variation in septal IB values might relate either to intrinsically greater variation in septal function compared with the free wall, greater difficulty in identifying a consistent region of interest within the interventricular septum, or more variation associated with anisotropy. Septal contractile function does not appear to differ appreciably from free wall function (Maier *et al.*, 1992),

making it unlikely that intrinsic contractile differences are responsible. Variation might relate to measurement difficulties, however. The effects of intervening blood and tissue with closed chest IB studies would lead one to expect the free wall measurements to be more variable, but this does not seem to be the case. However, near-field structures and right ventricular chordae tendineae in particular can complicate identification of the right ventricular endocardial component of the interventricular septum, which would result in greater tissue heterogeneity and increased variability of the cyclic variation results. Amplitude of cyclic variation is altered with orientation of myocardial fibres, and anisotropy could be an additional reason (Madaras *et al.*, 1988). Variance of cyclic variation has been shown to be higher in short axis and apical views than in parasternal long axis views (Vandenberg *et al.*, 1989).

#### *6.6.2.2 Cyclic variation in normal dogs*

The IB values in the normal dogs in this study varied in magnitude according to the phase of the cardiac cycle, in agreement with the findings in most other studies. The maximal value was found at or near the end-diastolic frame, and the minimal value was seen at or near the end-systolic frame. The amplitude of cyclic variation using the difference between end-diastolic and end-systolic values was also similar to the findings of other groups, as well as to other studies using this protocol (Lange *et al.*, 1995; Moran *et al.*, 1994). Other authors have also reported that the left ventricular free wall has greater amplitude of cyclic variation than that of the interventricular septum (Bouki *et al.*, 1996; Lange *et al.*, 1995; Masuyama *et al.*,

1989a), and this may reflect regional differences in contractile function (Haendchen *et al.*, 1983).

The method of calculation of cyclic variation significantly affected the results. Some studies have simply reported the difference between systolic and diastolic values (Barzilai *et al.*, 1984; Rijsterborgh *et al.*, 1991; Rijsterborgh *et al.*, 1990; Rijsterborgh *et al.*, 1993). The averaged maximum IB value minus the averaged minimum value has been used by others (Nozaki *et al.*, 1995; Wickline *et al.*, 1985b) or the peak-to-peak amplitude of the fundamental spectral component (Glueck *et al.*, 1985; Sagar *et al.*, 1990). The advantage of identifying maximum peak-to-trough values rather than using set time points such as end-diastole is that any phase shift in the cyclic variation relative to the cardiac cycle can also be calculated. The difference in systolic and diastolic values will be similar to the peak-to-trough value whenever the maximum value of IB occurs at end-diastole, but will be greatly affected if there is any phase shift in the cyclic variation, as has been shown to occur in abnormal myocardium (Barzilai *et al.*, 1990). Even in the normal dogs in the present study, there were significant differences in all the regions between the two methods of calculating cyclic variation.

More sophisticated methods of assessing cyclic variation have been developed. Although the fundamental frequency spectral component has been used as the basis for deriving the amplitude of cyclic backscatter, the variation in IB values does not actually follow a sinusoidal pattern, especially at low heart rates (Mohr *et al.*, 1989). Computer algorithms have been derived that make minimal assumptions about the form of the variation, and require only basic ECG timing

reference data points with the IB data (Mobley *et al.*, 1995; Mohr *et al.*, 1989). A model function is produced and low pass filtered, then offset to have zero mean over the cardiac period, and finally the data set is truncated at both ends. The least squares fit of the smoothed data is then subtracted from the filtered data. This algorithm is now available as a computer programme that can be downloaded from the website of the Laboratory for Ultrasonics, Washington University, St Louis. Unfortunately, this programme requires a minimum of one complete cardiac cycle, so that it was not possible to use it in the present study, where slightly less than one complete cardiac cycle was collected for many of the subjects.

### 6.6.3 Dogs with DCM

The number of affected dogs was less than ideal, with only four cocker spaniels to compare with the nine Dobermanns. In fact, there appeared to be little difference in the results between the Dobermanns and cocker spaniels, and it was probably appropriate to pool the results into one affected group. Unfortunately the number of scans for each affected dog was not consistent; seven dogs were only scanned once.

#### *6.6.3.1 Cyclic variation in dogs with DCM*

When cyclic variation was calculated as the difference between end-diastolic and end-systolic values, there were significantly lower values seen in the dogs with DCM compared with the normal dogs, but no significant differences were seen between the Dobermanns and cocker spaniels. This is consistent with the

reduced or absent cyclic variation that has typically been reported (Baysal *et al.*, 1991; Fujimoto *et al.*, 1999; Goens *et al.*, 1996; Naito *et al.*, 1996b; Vered *et al.*, 1987; Zuber *et al.*, 1999). However, some studies have noted that cyclic variation can be present in some DCM patients, and further refinements may be needed in order to identify IB abnormalities in these patients (Bouki *et al.*, 1996; Naito *et al.*, 1996a).

Despite the differences using the  $CV_{ED-ES}$  method, no significant differences were found between the normal dogs and the dogs with DCM when the peak-to-trough measurement of cyclic variation was used. One possible explanation is the lack of any measure of phase delay in either measurement method. The delay in cyclic variation may be a more sensitive measure of reduced contractile function than the absolute amplitude of variation (Lin *et al.*, 1998; Takiuchi *et al.*, 1998; Wagner *et al.*, 1995). A phase shift would result in a diminished end-diastolic to end-systolic value even when the overall amplitude of cyclic variation was preserved. A difference in “apparent” magnitude of cyclic variation (measured as end-diastolic and end-systolic values) and actual magnitude in cyclic variation was noted by Finch-Johnston and others in normal human subjects according to the region examined (Finch-Johnston *et al.*, 1999). Even minor phase shifts in some regions resulted in discrepancies between the apparent and actual magnitude of cyclic variation.

Another possible confounding factor is that although end-diastolic frames were identified from a concurrent ECG, a reduced left ventricular ejection time in the DCM patients (approximately 15 ms in the DCM dogs, compared with 20 ms in the normal dogs) would result in a different number of IB frames over the systolic

period. Without a more sophisticated analysis of the waveforms, the only practical means of comparing the normal and DCM groups was to pool the data over the number of frames, and it was not possible to make adjustments for individual dogs with shorter systolic periods without sacrificing information on the cyclic nature of the backscatter patterns. Use of the Washington University computer programme would have allowed incorporation of this factor into the comparison of the groups.

The normal dogs had significant regional variations in cyclic variation, with the septum showing less cyclic variation than the free wall. Such regional differences were not demonstrated in either DCM group, using either method of calculating cyclic variation. Bouki and others (1996) reported similar findings, with marked regional differences in cyclic variation in normal subjects, but very little regional variability in DCM patients. Loss of regional variability may be a more sensitive marker of LV dysfunction in DCM than reduced cyclic variation alone.

## 6.7 CONCLUSIONS

Integrated backscatter can be measured in the time-domain in closed-chest, conscious unsedated dogs, and appears to be adequately reproducible. Use of cyclic variation allows comparison of integrated backscatter characteristics between individuals, and from this study, it appears that normal dogs show cyclic variation in IB when regions in the LV free wall and septum are examined from a right parasternal long axis view. This CVIB shows a maximum in end-diastole, and a minimum in end-systole, in concordance with previous studies. In normal dogs, the magnitude of CVIB is greatest in the LV free wall, with lower values of CVIB in the interventricular septum.

Dogs with DCM and reduced myocardial function show reduced CVIB, as well as lack of regional variation in CVIB. Reduced CVIB was seen when defined as the difference between end-diastolic and end-systolic values, but not when calculated as the maximum amplitude of variation over the entire cardiac cycle. No regional variation was noted when either method of defining CVIB was used. Dogs with DCM may also have phase shifts in CVIB, although this was not specifically examined. No differences were found between the two groups of dogs affected with DCM, although the number of cocker spaniels was small.

Further studies should evaluate data from multiple cardiac cycles, and should include analysis of the phase delay of cyclic variation. Collection of IB values from multiple views might also improve the sensitivity of the technique, as could the use of a dobutamine stress technique.



## CHAPTER 7: GENERAL DISCUSSION

The ultrasound studies reported in this thesis clearly demonstrated differences in cardiac structure and function between normal dogs and dogs with dilated cardiomyopathy (DCM). Although a clinical diagnosis of DCM is often based on one or two key echocardiographic variables (LV fractional shortening, LV diameter), this study showed that many other echocardiographic indices may be abnormal in affected dogs. All the M-mode variables, all the 2D echocardiographic variables, and all but four of the Doppler echocardiographic variables examined were significantly different in the dogs with DCM. At the same time, this study showed that clinically normal dogs may have M-mode measurements that overlap with dogs with DCM. In particular, fractional shortening may fall outside commonly used reference ranges in normal dogs, and excessive reliance on this one variable may lead to an erroneous diagnosis of DCM. This study showed that there is a wide choice of echocardiographic measurements that may be abnormal in dogs with DCM, and the specificity of echocardiography as a screening test for DCM could be improved if multiple variables were taken into account. If one variable only were to be used, this study demonstrated that LV ejection fraction showed the clearest separation between normal dogs and dogs with DCM, and this variable has the advantage of being bodyweight-independent.

Clear differences were seen in outcome between the two affected breeds. As expected, the Dobermanns had much shorter survival times than the cocker spaniels. An aim of this study was to use echocardiography to identify differences in chamber size, or in systolic and diastolic function between these two groups of dogs,

which might provide some clue to prognosis. Many echocardiographic variables have been shown to be prognostic indicators, and a range of echocardiographic measurements were made in the two breeds. Most of the variables were astonishingly similar in the two breeds, once the confounding influence of body size was accounted for. None of the M-mode or 2D echocardiographic measurements showed significant differences between the two groups, apart from differences relating to bodyweight.

In contrast, not only were there significant differences in three Doppler variables, but these were independent of bodyweight, and two of the three (mitral inflow E/A ratio, isovolumic relaxation time) coincided with key variables that have been linked to prognosis in human DCM. The dogs could be classified on the basis of their transmitral flow profiles, including information on the relative contribution of early to late transmitral flow, isovolumic relaxation time, and mitral deceleration time. Even without a more sophisticated classification system for categorising diastolic function using pulmonary venous flow patterns, Dobermanns showed a predominantly restrictive pattern of filling, and the cocker spaniels showed a range of transmitral flow that included an abnormal relaxation pattern. In countless human studies, a restrictive pattern of filling has been associated with shorter survival times, and abnormal relaxation patterns have been linked to a better prognosis. Although all patients are suspected of having abnormal left ventricular relaxation, this is masked by increased left atrial driving pressure and reduced ventricular compliance in the patients with a restrictive pattern of filling. The same pathophysiological processes could account for the differences in outcome between Dobermanns and cocker spaniels.

The relation to outcome in people is improved further if the transmitral flow pattern after therapy is included (Pinamonti *et al.*, 1997; Traversi *et al.*, 1996). The effect of therapy on transmitral flow pattern was not examined in these dogs, but is certainly worth further evaluation.

The integrated backscatter (IB) studies reported in this thesis show that it is technically feasible to measure IB in conscious dogs, and that normal dogs show reproducible cyclic variation in IB. The dogs with DCM did not show similar patterns of cyclic variation, although significant differences were not found with the normal dogs unless the end-diastolic to end-systolic variation was measured. The affected dogs showed a similar range of IB levels over the cardiac cycle as the normal dogs, but the pattern was not as consistent. Ideally, the phase delay of the cyclic variation should also have been examined in the abnormal dogs. The statistical analysis was complicated by the cyclic nature of the variable, and the small number of cardiac cycles examined.

## 7.1 STUDY LIMITATIONS

As with many clinical studies, the number of animals examined was small. This hampered attempts at regression analysis, so that the prognostic value of individual variables could not be easily assessed. Instead, this study could be viewed as a pilot study, identifying potentially useful variables for subsequent testing in a prospective manner. This study has been useful in indicating that transmitral flow patterns would be worth pursuing, and suggests that other measurements of diastolic function (such as pulmonary venous flow patterns) might also be usefully included.

The reproducibility of some of the Doppler variables was poor. The Vingmed system used had comparatively slow sweep speeds, and this made measurement of time intervals imprecise. Reproducibility would probably have been improved if recordings had been made at faster sweep speeds.

In the IB studies, recording data from greater numbers of cardiac cycles would have been very helpful. The time-consuming nature of the data acquisition limited the number of scans that could be collected, and it was impractical to record more than two scans in one session using client-owned dogs. At the time of recording, it was only possible to obtain information on phase delay in individual dogs by acquiring continuous data. Subsequently, computer analysis has become available that would allow calculation of phase delay with collection of relatively few frames per cardiac cycle, and it now transpires that it would have been preferable to acquire data from frames at key points of the cardiac cycle triggered from the ECG.

## 7.2 FURTHER STUDIES

More refined analysis of IB measurements will be required before the clinical utility of IB is shown in the assessment of DCM in dogs. Larger number of dogs, and collection of data from greater numbers of cardiac cycles should prove helpful in determining the value of phase delay of cyclic variation.

The transmitral flow variables highlighted in this study as potentially useful prognostic indicators should be applicable to other breeds affected with DCM, and possibly other causes of heart failure. Further studies would be indicated in other breeds with DCM. It would also be useful to examine the effect of therapy on

diastolic filling variables. At present, the prognosis in canine DCM patients is based on a few reports in the literature associating severe congestive failure (pleural effusion or biventricular heart failure) with poor outcome (Calvert *et al.*, 1997; Monnet *et al.*, 1995), or anecdotal evidence related to breed or response to treatment. Transmitral flow is readily assessed with Doppler echocardiography, and may prove to be a practical, objective and clinically useful variable in the assessment of prognosis in dogs with DCM.

## Appendix 1

### MODIFIED NEW YORK HEART ASSOCIATION (NYHA) CLASSIFICATION

<b>NYHA Class 1</b>	Evidence of cardiac disease, but no limitations of physical activity.
<b>NYHA Class 2</b>	Evidence of cardiac disease, but no clinical signs except at exercise (i.e. slight exercise intolerance). Comfortable at rest.
<b>NYHA Class 3</b>	Cardiac disease associated with clinical signs such as coughing or dyspnoea associated with congestive heart failure, but animal still able to function normally at rest.
<b>NYHA Class 4</b>	Cardiac disease associated with severe congestive heart failure, such that the animal is compromised even at rest.

Adapted from: (The Criteria Committee of the New York Heart Association, 1973)

## Appendix 2

### VOLUME MEASUREMENTS USING AREA-LENGTH METHOD

Table A2.1: Two-dimensional echocardiographic LV volume measurements in GSH pointers using Area-Length method.

Dog	EDV mL	EDVI mL/m <sup>2</sup>	ESV mL	ESVI mL/m <sup>2</sup>	SV mL	SVI mL/m <sup>2</sup>	EF% %
1	83.31	94.67	37.13	42.19	46.18	52.48	55.4
2	103.19	114.66	*46.8	*52.00	56.39	62.66	54.6
3	99.95	98.96	39.89	39.50	60.06	59.47	60.1
4	91.02	90.12	35.54	35.19	55.48	54.93	61.0
5	91.07	103.49	33.55	38.13	57.52	65.36	63.2
6	82.06	105.21	28.33	36.32	53.73	68.88	65.5
7	61.53	74.13	23.11	27.84	38.42	46.29	62.4
8	62.12	73.08	23.5	27.65	38.62	45.44	62.2
9	60.6	64.47	22.91	24.37	37.69	40.10	62.2
10	83.94	79.94	30.07	28.64	53.87	51.30	64.2
11	79.98	98.74	35.71	44.09	44.27	54.65	55.4
12	71.32	88.05	22.09	27.27	49.23	60.78	69.0
13	78.99	89.76	29.29	33.28	49.7	56.48	62.9
mean	80.70	90.41	31.38	35.11	49.32	55.29	61.39
SD	13.90	14.41	7.57	8.04	7.72	8.28	4.18

\*indicates value >2SD from the mean. LV: left ventricular, GSH: German Short-Haired, EDV: left ventricular end-diastolic volume, EDVI: EDV normalised for body surface area, ESV: left ventricular end-systolic, ESVI: ESV normalised for body surface area, SV: left ventricular stroke volume, SV index: SV normalised for body surface area. EF%: left ventricular ejection fraction, SD: standard deviation, NR: not recorded.

Table A2.2: Repeated two-dimensional echocardiographic LV volume measurements in GSH pointers using Area-Length method.

	A-L EDV (mL)		A-L ESV (mL)		A-L SV (mL)		A-L EF (%)	
	Dog 1	Dog 2	Dog 1	Dog 2	Dog 1	Dog 2	Dog 1	Dog 2
Day 1	80.3	79.0	25.7	29.3	54.6	49.7	68.0	62.92
Day 2	71.3	80.3	22.1	31.3	49.2	49.0	69.0	61.01
Day 3	80.0	76.9	30.3	28.0	49.7	49.0	62.1	63.66
Day 4	79.2	61.8	28.5	25.8	50.7	36.0	64.0	58.21
Day 5	77.3	76.9	31.2	27.6	46.1	49.4	59.7	64.17
mean	77.6	75.0	27.6	28.4	50.1	46.6	64.6	61.99
SD	3.7	7.5	3.7	2.0	3.1	6.0	3.9	2.43
CV%	4.8	10.0	13.4	7.2	6.2	12.78	6.12	3.9

LV: left ventricle, GSH: German short-haired, D.: diastolic, S.: systolic, EDV: left ventricular end-diastolic volume, ESV: left ventricular end-systolic volume, SV: left ventricular stroke volume, EF: left ventricular ejection fraction, SD: standard deviation, CV%: coefficient of variation.



Table A2.3: 2DE LV volume measurements in Dobermanns using Area-Length method.

Dog	EDV mL	EDVI mL/m <sup>2</sup>	ESV mL	ESVI mL/m <sup>2</sup>	SV mL	SVI mL/m <sup>2</sup>	EF% %
1	168.78	165.47	126.37	123.89	42.41	41.58	25.1
2	121.74	126.81	84.44	87.96	37.3	38.85	30.6
3	110.05	114.64	84.1	87.60	25.95	27.03	23.6
4	105.61	75.44	88.13	62.95	17.48	12.49	16.6
5	NR	NR	NR	NR	NR	NR	NR
6	204.04	182.18	154.58	138.02	49.46	44.16	24.2
7	174.4	169.32	148.82	144.49	25.58	24.83	14.7
8	189.1	178.40	149.58	141.11	39.52	37.28	20.9
9	137.95	140.77	99.39	101.42	38.56	39.35	28.0
10	147.81	115.48	117.14	91.52	30.67	23.96	20.7
11	183.7	157.01	152.8	130.60	30.9	26.41	16.8
Mean	154.32	142.55	120.54	110.96	33.78	31.59	22.12
SD	34.74	34.28	29.85	28.22	9.43	10.11	5.17

\*Indicates value >2SD from the mean. LV: left ventricular, EDV: left ventricular end-diastolic volume, EDVI: EDV normalised for body surface area, ESV: left ventricular end-systolic, ESVI: ESV normalised for body surface area, SV: left ventricular stroke volume, SV index: SV normalised for body surface area. EF%: left ventricular ejection fraction, SD: standard deviation, NR: not recorded.

Table A2.4: 2DE LV volume measurements in Cocker spaniels using Area-Length method.

Dog	EDV	EDVI	ESV	ESVI	SV	SVI	EF%
	mL	mL/m <sup>2</sup>	mL	mL/m <sup>2</sup>	mL	mL/m <sup>2</sup>	%
1	82.94	140.58	70.89	120.15	12.05	20.42	14.5
2	113.6	218.46	85.5	164.42	28.1	54.04	24.7
3	108.13	171.63	94.25	149.60	13.88	22.03	12.8
4	NR	NR	NR	NR	NR	NR	NR
5	44.66	69.78	32.6	50.94	12.06	18.84	27.0
6	99.39	139.99	76.92	108.34	22.47	31.65	22.6
7	135.3	225.50	110.39	183.98	24.91	41.52	18.4
8	NR	NR	NR	NR	NR	NR	NR
9	117.2	189.03	85.57	138.02	31.63	51.02	27.0
10	NR	NR	NR	NR	NR	NR	NR
11	NR	NR	NR	NR	NR	NR	NR
Mean	100.17	165.00	79.45	130.78	20.73	34.22	21.02
SD	29.29	53.90	24.24	43.51	8.07	14.78	5.83

LV: left ventricular, EDV: left ventricular end-diastolic volume, EDVI: EDV normalised for body surface area, ESV: left ventricular end-systolic, ESVI: ESV normalised for body surface area, SV: left ventricular stroke volume, SV index: SV normalised for body surface area. EF%: left ventricular ejection fraction, SD: standard deviation, NR: not recorded.

## Appendix 3

### AORTIC MEASUREMENTS FROM SUBCOSTAL VIEW

Table A3: Doppler measurements of subcostal aortic flow in individual normal German short-haired pointers.

Dog	Subcostal window							
	Ao vel ms <sup>-1</sup>	VTI cm	HR /min	dV/dt ms <sup>-2</sup>	acc dt s	ET s	PEP s	PEP/ET
1	1.33	14.01	78.3	29.41	0.047	0.19	0.061	0.321
2	1.31	15.11	61.2	24.14	0.057	0.201	0.064	0.318
3	1.76	19.6	84.7	31.54	0.06	0.199	0.059	0.296
4	1.15	10.85	117.7	23.34	0.053	*0.143	0.0625	*0.437
5	1.65	14.81	96.4	40.67	0.043	0.165	0.048	0.291
6	NR	NR	NR	NR	NR	NR	NR	NR
7	NR	NR	NR	NR	NR	NR	NR	NR
8	1.32	12.25	115.1	44.1	0.033	0.173	0.069	0.399
9	1.26	12.9	96.3	26.33	0.038	0.2	0.054	0.270
10	NR	NR	NR	NR	NR	NR	NR	NR
11	1.08	11.09	67.8	19.22	0.061	0.189	0.061	0.323
12	1.62	17.24	78.4	31.2	0.057	0.193	0.052	0.269
13	1.56	16.77	82.2	37.38	0.043	0.194	0.059	0.304
mean	1.40	14.46	87.81	30.73	0.05	0.18	0.06	0.323
SD	0.23	2.83	18.60	7.99	0.01	0.02	0.01	0.054

\*Indicates value >2SD from the mean. GSH: German Short-Haired, Ao vel: aortic velocity, VTI: velocity time integral, HR: heart rate, dV/dt: aortic flow acceleration, acc dt: aortic flow acceleration time, ET: left ventricular ejection time, PEP: left ventricular pre-ejection period, SD: standard deviation, NR: not recorded.

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## **APPENDIX 1**

### **MODIFIED NEW YORK HEART ASSOCIATION (NYHA) CLASSIFICATION**

<b>NYHA Class 1</b>	Evidence of cardiac disease, but no limitations of physical activity.
<b>NYHA Class 2</b>	Evidence of cardiac disease, but no clinical signs except at exercise (i.e. slight exercise intolerance). Comfortable at rest.
<b>NYHA Class 3</b>	Cardiac disease associated with clinical signs such as coughing or dyspnoea associated with congestive heart failure, but animal still able to function normally at rest.
<b>NYHA Class 4</b>	Cardiac disease associated with severe congestive heart failure, such that the animal is compromised even at rest.

Adapted from: {The Criteria Committee of the New York Heart Association 1973 ID: 5932}

## APPENDIX 2

**Table A2.1: Two-dimensional echocardiographic LV volume measurements in GSH pointers using Area-Length method.**

2.1.1.1	EDV	EDVI	ESV	ESVI	SV	SVI	EF%
2.1.1.2	mL	mL/m <sup>2</sup>	mL	mL/m <sup>2</sup>	mL	mL/m <sup>2</sup>	%
1	83.31	94.67	37.13	42.19	46.18	52.48	55.4
2	103.19	114.66	*46.8	*52.00	56.39	62.66	54.6
3	99.95	98.96	39.89	39.50	60.06	59.47	60.1
4	91.02	90.12	35.54	35.19	55.48	54.93	61.0
5	91.07	103.49	33.55	38.13	57.52	65.36	63.2
6	82.06	105.21	28.33	36.32	53.73	68.88	65.5
7	61.53	74.13	23.11	27.84	38.42	46.29	62.4
8	62.12	73.08	23.5	27.65	38.62	45.44	62.2
9	60.6	64.47	22.91	24.37	37.69	40.10	62.2
10	83.94	79.94	30.07	28.64	53.87	51.30	64.2
11	79.98	98.74	35.71	44.09	44.27	54.65	55.4
12	71.32	88.05	22.09	27.27	49.23	60.78	69.0
13	78.99	89.76	29.29	33.28	49.7	56.48	62.9
mean	80.70	90.41	31.38	35.11	49.32	55.29	61.39
SD	13.90	14.41	7.57	8.04	7.72	8.28	4.18

\*indicates value >2SD from the mean. LV: left ventricular, GSH: German Short-Haired, EDV: left ventricular end-diastolic volume, EDVI: EDV normalised for body surface area, ESV: left ventricular end-systolic, ESVI: ESV normalised for body surface area, SV: left ventricular stroke volume, SV index: SV normalised for body surface area. EF%: left ventricular ejection fraction, SD: standard deviation, NR: not recorded.



**Table A2.2: Repeated two-dimensional echocardiographic LV volume measurements in GSH pointers using Area-Length method.**

	A-L EDV (mL)		A-L ESV (mL)		A-L SV (mL)		A-L EF (%)	
	Dog 1	Dog 2	Dog 1	Dog 2	Dog 1	Dog 2	Dog 1	Dog 2
Day 1	80.3	79.0	25.7	29.3	54.6	49.7	68.0	62.92
Day 2	71.3	80.3	22.1	31.3	49.2	49.0	69.0	61.01
Day 3	80.0	76.9	30.3	28.0	49.7	49.0	62.1	63.66
Day 4	79.2	61.8	28.5	25.8	50.7	36.0	64.0	58.21
Day 5	77.3	76.9	31.2	27.6	46.1	49.4	59.7	64.17
mean	77.6	75.0	27.6	28.4	50.1	46.6	64.6	61.99
SD	3.7	7.5	3.7	2.0	3.1	6.0	3.9	2.43
CV%	4.8	10.0	13.4	7.2	6.2	12.78	6.12	3.9

LV: left ventricle, GSH: German short-haired, D.: diastolic, S.: systolic, EDV: left ventricular end-diastolic volume, ESV: left ventricular end-systolic volume, SV: left ventricular stroke volume, EF: left ventricular ejection fraction, SD: standard deviation, CV%: coefficient of variation.

**Table A2.3: 2DE LV volume measurements in Dobermanns using Area-Length method.**

2.1.1.3	EDV	EDVI	ESV	ESVI	SV	SVI	EF%
2.1.1.4	mL	mL/m <sup>2</sup>	mL	mL/m <sup>2</sup>	mL	mL/m <sup>2</sup>	%
1	168.78	165.47	126.37	123.89	42.41	41.58	25.1
2	121.74	126.81	84.44	87.96	37.3	38.85	30.6
3	110.05	114.64	84.1	87.60	25.95	27.03	23.6
4	105.61	75.44	88.13	62.95	17.48	12.49	16.6
5	NR	NR	NR	NR	NR	NR	NR
6	204.04	182.18	154.58	138.02	49.46	44.16	24.2
7	174.4	169.32	148.82	144.49	25.58	24.83	14.7
8	189.1	178.40	149.58	141.11	39.52	37.28	20.9
9	137.95	140.77	99.39	101.42	38.56	39.35	28.0
10	147.81	115.48	117.14	91.52	30.67	23.96	20.7
11	183.7	157.01	152.8	130.60	30.9	26.41	16.8
Mean	154.32	142.55	120.54	110.96	33.78	31.59	22.12

SD	34.74	34.28	29.85	28.22	9.43	10.11	5.17
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\*Indicates value >2SD from the mean. LV: left ventricular, EDV: left ventricular end-diastolic volume, EDVI: EDV normalised for body surface area, ESV: left ventricular end-systolic, ESVI: ESV normalised for body surface area, SV: left ventricular stroke volume, SV index: SV normalised for body surface area. EF%: left ventricular ejection fraction, SD: standard deviation, NR: not recorded.

**Table A2.4: 2DE LV volume measurements in Cocker spaniels using Area-Length method.**

2.1.1.5	EDV	EDVI	ESV	ESVI	SV	SVI	EF%
2.1.1.6	mL	mL/m <sup>2</sup>	mL	mL/m <sup>2</sup>	mL	mL/m <sup>2</sup>	%
1	82.94	140.58	70.89	120.15	12.05	20.42	14.5
2	113.6	218.46	85.5	164.42	28.1	54.04	24.7
3	108.13	171.63	94.25	149.60	13.88	22.03	12.8
4	NR	NR	NR	NR	NR	NR	NR
5	44.66	69.78	32.6	50.94	12.06	18.84	27.0
6	99.39	139.99	76.92	108.34	22.47	31.65	22.6
7	135.3	225.50	110.39	183.98	24.91	41.52	18.4
8	NR	NR	NR	NR	NR	NR	NR
9	117.2	189.03	85.57	138.02	31.63	51.02	27.0
10	NR	NR	NR	NR	NR	NR	NR
11	NR	NR	NR	NR	NR	NR	NR
Mean	100.17	165.00	79.45	130.78	20.73	34.22	21.02
SD	29.29	53.90	24.24	43.51	8.07	14.78	5.83

LV: left ventricular, EDV: left ventricular end-diastolic volume, EDVI: EDV normalised for body surface area, ESV: left ventricular end-systolic, ESVI: ESV normalised for body surface area, SV: left ventricular stroke volume, SV index: SV normalised for body surface area. EF%: left ventricular ejection fraction, SD: standard deviation, NR: not recorded.

**Table A3: Doppler measurements of subcostal aortic flow in individual normal German short-haired pointers.**

2.1.1.7	Subcostal window							
2.1.1.8	Ao vel ms <sup>-1</sup>	VTI cm	HR /min	dV/dt ms <sup>-2</sup>	acc dt s	ET s	PEP s	PEP/ET
1	1.33	14.01	78.3	29.41	0.047	0.19	0.061	0.321
2	1.31	15.11	61.2	24.14	0.057	0.201	0.064	0.318
3	1.76	19.6	84.7	31.54	0.06	0.199	0.059	0.296
4	1.15	10.85	117.7	23.34	0.053	*0.143	0.0625	*0.437
5	1.65	14.81	96.4	40.67	0.043	0.165	0.048	0.291
6	NR	NR	NR	NR	NR	NR	NR	NR
7	NR	NR	NR	NR	NR	NR	NR	NR
8	1.32	12.25	115.1	44.1	0.033	0.173	0.069	0.399
9	1.26	12.9	96.3	26.33	0.038	0.2	0.054	0.270
10	NR	NR	NR	NR	NR	NR	NR	NR
11	1.08	11.09	67.8	19.22	0.061	0.189	0.061	0.323
12	1.62	17.24	78.4	31.2	0.057	0.193	0.052	0.269
13	1.56	16.77	82.2	37.38	0.043	0.194	0.059	0.304
mean	1.40	14.46	87.81	30.73	0.05	0.18	0.06	0.323
SD	0.23	2.83	18.60	7.99	0.01	0.02	0.01	0.054

\*Indicates value >2SD from the mean. GSH: German Short-Haired, Ao vel: aortic velocity, VTI: velocity time integral, HR: heart rate, dV/dt: aortic flow acceleration, acc dt: aortic flow acceleration time, ET: left ventricular ejection time, PEP: left ventricular pre-ejection period, SD: standard deviation, NR: not recorded.

# Measurement of cyclic variation in ultrasonic integrated backscatter in conscious, unsedated, clinically normal dogs

Virginia Luis Fuentes, VetMB; Carmel M. Moran, BSc, PhD; Karsten Schober, DVM;  
Joanna Dukes McEwan, BVMS, MVM; Helen Brown, BA, MSc; George R. Sutherland, MBChB, MRCP;  
W. Norman McDicken, BSc, PhD

**Objective**—To assess the feasibility and repeatability of measuring ultrasonic integrated backscatter in unsedated conscious dogs, using a protocol previously validated in pigs with open thorax.

**Animals**—11 clinically normal conscious unsedated German Shorthair Pointers.

**Procedure**—A modified commercially available echocardiography system was used to record long-axis views of the heart. The radiofrequency data from 15 consecutive frames were digitized and analyzed. Regions of interest were chosen within the myocardium, and the ultrasonic integrated backscatter within each region was calculated in the time domain for each frame.

**Results**—Cyclic variation in integrated backscatter values was observed, with maximal values at end-diastole and minimal values at end-systole. Mean  $\pm$  SD amplitude of cyclic variation was  $5.81 \pm 3.86$  dB over all the regions chosen.

**Conclusions**—Results agreed with those obtained by other investigators working with dogs with open thorax and those with closed thorax while under general anesthesia. The analysis of the components of variance indicates that this is a consistent, reliable technique in conscious unsedated dogs.

**Clinical Relevance**—Integrated ultrasonic backscatter measurement provides a noninvasive means of tissue characterization. Use of this protocol reliably yields cyclic variation in integrated backscatter and could be applied clinically to dogs with myocardial disease. (*Am J Vet Res* 1997;58:1055-1059)

The interaction of ultrasound waves with biological tissue is strongly influenced by the physical characteristics of the tissue, and this interaction can be exploited to provide a noninvasive method of ultrasonic

tissue characterization. Ultrasonic tissue characterization techniques have been documented to be capable of distinguishing normal from ischemic myocardium in studies of dogs with open thorax and in human patients,<sup>1,2</sup> and normal myocardium from that of human beings with dilated cardiomyopathy,<sup>3</sup> hypertrophic cardiomyopathy,<sup>4</sup> and diabetic myocardial disease.<sup>5</sup>

A number of methods have been described for the quantification of myocardial ultrasonic reflectivity by measuring small amplitude radiofrequency (RF) signals. However, techniques vary among laboratories, and there is no standard in vivo technique against which other techniques can be compared. Integrated backscatter (IB) is the most commonly measured in vivo variable, although a number of calculations have been used to derive IB, including frequency domain<sup>6</sup> and time domain techniques.<sup>7</sup> A cyclic variation with the phase of the cardiac cycle has been a consistent finding in myocardial IB, with maximal reflectivity generally corresponding to end-diastole. The magnitude of this cyclic variation has been found to vary with the myocardial site studied,<sup>8</sup> the angle of insonification, and left ventricular function. Most experimental studies have used dogs or pigs with open thorax. Of the few studies in which IB in dogs with closed thorax has been measured, anesthetized mixed breed dogs have generally been used.<sup>9-11</sup> To the authors' knowledge, reports of the measurement of IB in conscious, unsedated dogs do not exist.

The objective of the study reported here was to assess the feasibility of measuring ultrasonic IB from transthoracic views in conscious, unsedated dogs, using the protocol described by Moran et al<sup>12</sup> in pigs with open thorax. We also aimed to examine subject variation when a single breed of dog was used, and to measure intradog, interdog, and interobserver variability.

## Materials and Methods

**Dogs**—Eleven German Shorthair Pointers (8 females, 3 males) were studied. Mean age was 6.0 (range, 1.5 to 12) years, and mean ( $\pm$  SD) body weight was 25.6 ( $\pm$  3.5) kg. All dogs were considered to be healthy on the basis of historical findings and physical examination. All dogs were subjected to a routine two-dimensional, M-mode and Doppler echocardiographic examination to rule out any evidence of cardiac disease. All animals were used in accordance with the regulations and guidelines laid down in the UK Animals (Scientific Procedures) Act, 1986.

**Imaging system and RF data acquisition**—A modified commercially available digital echocardiography system<sup>a</sup> with

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From the Department of Veterinary Clinical Studies, Royal (Dick) School of Veterinary Studies, University of Edinburgh, Summerhall (Luis Fuentes, Schober, McEwan); Medical Statistics Unit, Department of Public Health Sciences, University of Edinburgh Medical School, Teviot Place (Brown); the Department of Cardiology, Western General Hospital, Crewe Rd (Sutherland); and the Department of Medical Physics and Medical Engineering, University of Edinburgh, Royal Infirmary (Moran, McDicken), Edinburgh, United Kingdom. Dr. Luis Fuentes' present address is the Department of Veterinary Medicine and Surgery, College of Veterinary Medicine and Surgery, Clydesdale Hall, University of Missouri-Columbia, Columbia, MO 65211.

a 5 MHz phased array transducer (bandwidth, 2.5 to 7.5 MHz), and simultaneous ECG was used to acquire the two-dimensional echocardiographic images (Fig 1). Dogs were positioned in right lateral recumbency on a modified table, to allow placement of the transducer on the thoracic wall from below. Right parasternal long-axis views were used to obtain images of the left ventricle. Transmit power and time gain compensation controls were set at preadjusted levels for the studies, and the gain was adjusted at the start of each study, then was kept constant. The RF signals were digitized at 12 MHz to 16 bit. Fifteen consecutive frames of RF data were collected, and manually controlled so that the first frame occurred shortly before end-diastole. Each echo line of RF data consisted of a maximum of 4,096 samples, depending on the image depth. A single image consisted of 128 lines with up to 4,096 samples/line at 2 bytes/sample, occupying 1 megabyte (MB) of storage memory/frame. The RF acquisition system was equipped with a 16-MB memory board, of which 1 MB was reserved for control registers, so that a maximum of 15 complete frames of RF data could be acquired. The two-dimensional images were also stored on video, and hard copies were made of each frame.

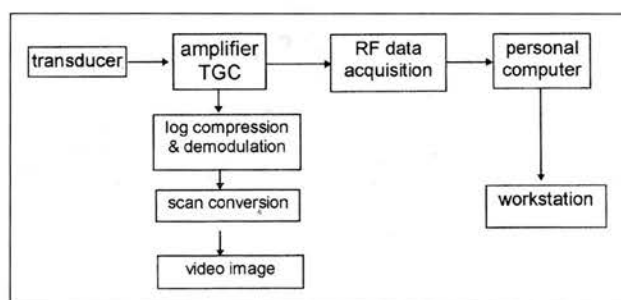


Figure 1—Block diagram showing the system used for the acquisition of radiofrequency (RF) data. TGC = time gain compensation.

The RF data were collected from each scan and downloaded onto a workstation<sup>b</sup> for signal processing. The RF data were rectified, low-pass filtered, logarithmically compressed, and scan converted before the reconstruction of two-dimensional images similar to the images obtained by the original echocardiography machine. Myocardial regions of interest in the reconstructed images were chosen within the left ventricular free wall and interventricular septum, using a mouse-driven cursor (Fig 2). Care was taken to avoid the specular echoes of the endocardial and epicardial surfaces. Regions of interest in the same myocardial area were tracked through each frame. These data were then related to the original uncompressed RF data, and the IB value was calculated. A set of calibration scans was also recorded, using a standard grayscale test tissue phantom to compensate for the effects of transducer characteristics, time gain compensation, depth, and dynamic range settings of the ultrasound machine. The calibration scans were recorded for each depth and focus setting used during the in vivo scans, and the RF data were downloaded to the workstation in similar manner. The regions of interest chosen for each canine scan were then superimposed on the phantom scan RF data, and the IB was calculated according to the following formula:

$$IB = \frac{\int_{t-\Delta t}^{t+\Delta t} |V(t)|^2 \delta t}{\int_{t-\Delta t}^{t+\Delta t} |P(t)|^2 \delta t}$$

where  $V(t)$  is the amplitude of the uncompressed RF signal from the myocardial region of interest,  $P(t)$  is the amplitude of the uncompressed RF signal from the phantom,  $\delta t$  is a small

increment of time over the timegate, and  $2\Delta t$  is the timegate over the region of interest.

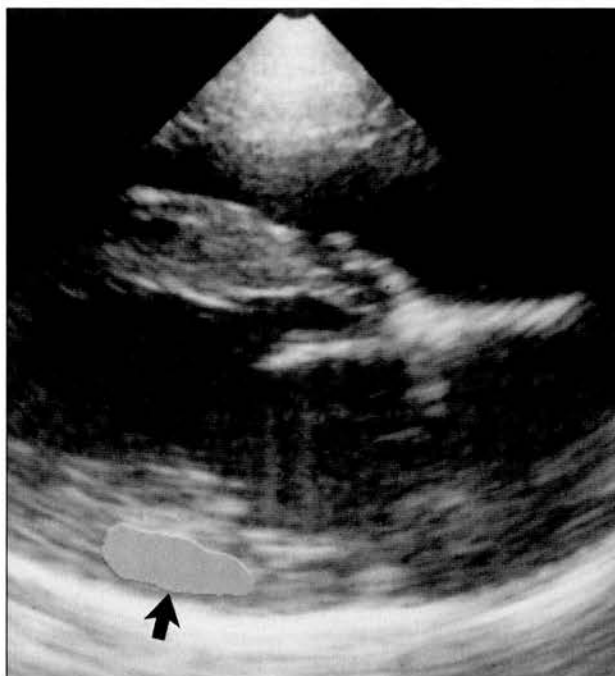


Figure 2—Reconstructed image on the workstation, showing region of interest chosen in the left ventricular free wall (arrow).

**Protocol**—All 11 dogs were scanned at least twice, and for each scan, values for IB were obtained for 3 myocardial regions over 15 frames (the basal left ventricular free wall, the apical left ventricular free wall, and the interventricular septum). In this manner, a set of IB values over 15 frames for each cardiac cycle was acquired in 86 sites. Ten scans from 8 dogs were analyzed by 3 independent observers over 3 regions, yielding 90 data sets comprising 15 frames for each data set. Three dogs were scanned 4 times, and 1 dog was scanned 3 times.

**Analysis of data**—The logarithm of each recorded IB value was multiplied by 10, and the mean value was subtracted to yield the variation about the mean, so that the mean value was designated as 0 dB. Using the concurrently recorded ECG, the 15 frames of RF data were synchronized for each scan by defining the end-diastolic frame as the frame recorded at the start of the R wave, and the end-systolic frame as the frame with the smallest left ventricular volume. The amplitude of cyclic variation was defined as the difference between the maximal and minimal values.

**Statistical analysis**—A random effects model was used to obtain variance component estimates for intradog, interdog, and interobserver variation within scans and within dogs. It was also used to obtain best least squares means and 95% confidence intervals. All calculations were carried out, using statistical analysis software.<sup>c</sup>

## Results

Mean IB values for each frame from all the scans in all the regions of interest were obtained (Fig 3 top left), with the associated 95% confidence limits. Mean backscatter values and 95% confidence intervals for the individual regions (basal left ventricular free wall, apical left ventricular free wall, and interventricular septum)



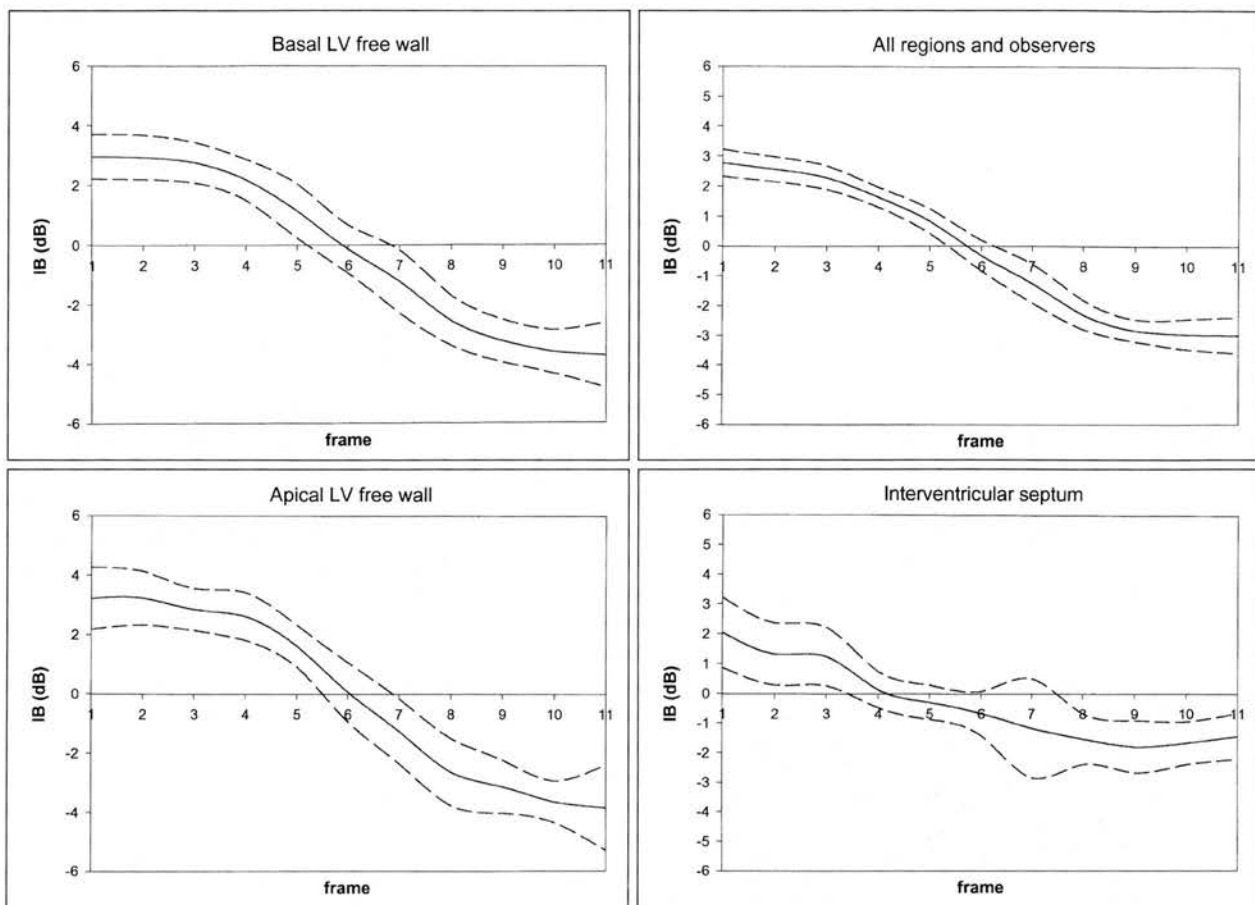


Figure 3—Mean integrated backscatter (IB) over 11 frames (from end-diastolic frame to end-systolic frame) for all scans and all observers. Mean IB levels for all 3 regions of interest and all 3 observers in all scans with 95% confidence intervals (top left). The same variables for the basal portion of the left ventricular (LV) free wall only (top right), apical portion of the LV free wall (bottom left), and the interventricular septum (bottom right).

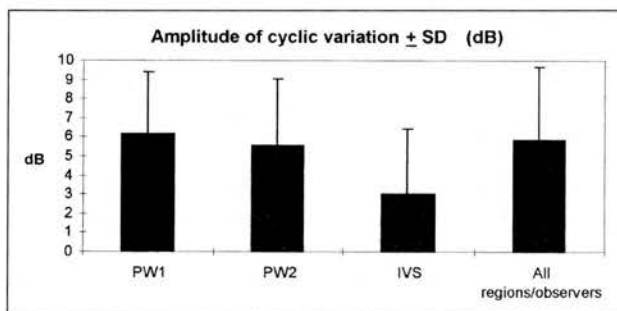


Figure 4—Mean  $\pm$  SD amplitude of cyclic variation in basal region of the left ventricular free wall (PW1), apical region of left ventricular free wall (PW2), and interventricular septum (IVS).

also were determined (Fig 3 top right, bottom left, and bottom right). Amplitude of cyclic variation in each of the various regions was compared (Fig 4) and was greatest in the basal portion of the left ventricular free wall (PW1), with a mean  $\pm$  SD value of  $6.17 \pm 3.19$  dB. The apical portion of the left ventricular free wall was similar ( $5.55 \pm 3.5$  dB), but the interventricular septum had a lesser degree of cyclic variation ( $3.04 \pm 3.4$  dB). The variance components for interdog, intradog, and interobserver variation for each frame were compared (Table 1). The intradog variance ranged from  $0.94 \text{ dB}^2$  for end-diastole to  $1.08 \text{ dB}^2$  for end-systole, decreasing to approximately zero at mid-systole. Inter-

dog variance was lowest at end-diastole ( $0.45 \text{ dB}^2$ ) and end-systole ( $0.78 \text{ dB}^2$ ), and highest at mid-systole ( $1.75 \text{ dB}^2$ ). Variance components attributable to different observers (whether within each dog or within each scan) were smaller, reaching a maximum of  $0.54 \text{ dB}^2$  for observer variance within each scan at end-systole. Other values are expressed as mean  $\pm$  SD unless otherwise stated.

## Discussion

Ultrasonic tissue characterization can provide unique insight into structural and functional changes in the myocardium, without recourse to invasive techniques, such as endomyocardial biopsy. Integrated backscatter measurement has been found to differentiate viable "stunned" ischemic myocardium from non-viable ischemic myocardium within 15 minutes of coronary occlusion in dogs before return to normal of any conventional echocardiographic variables, such as percentage of wall thickening.<sup>1</sup> Integrated backscatter has also been documented to differentiate hypertrophy attributable to athleticism from hypertrophic cardiomyopathy,<sup>13</sup> as well as hypertrophic cardiomyopathy from hypertrophy secondary to systemic hypertension.<sup>14</sup> A cyclic variation in IB, corresponding with the cardiac cycle, has been one of the most consistent findings in studies measuring IB in contracting myocardium.<sup>9,15-23</sup> The precise mechanism of this cyclic variation is unknown. It has been proposed that the intracellular and

Table 1—Variance components for integrated backscatter (IB) in all regions and all observers

Frame	Integrated backscatter (dB)		Intradog variation		Observer variation within each dog		Observer variation within each scan		Interdog variation	
	Mean	SEM	Variance (dB <sup>2</sup> )	SD (dB)	Variance (dB <sup>2</sup> )	SD (dB)	Variance (dB <sup>2</sup> )	SD (dB)	Variance (dB <sup>2</sup> )	SD (dB)
End-diastolic	2.78	0.23	0.94	0.97	0.00	0.00	0.43	0.66	0.43	0.66
2	2.56	0.21	0.85	0.92	0.12	0.35	0.22	0.47	0.65	0.81
3	2.28	0.2	0.49	0.70	0.21	0.46	0.07	0.26	0.96	0.98
4	1.68	0.17	0.32	0.57	0.04	0.20	0.27	0.52	0.44	0.66
5	0.83	0.21	0.00	0.00	0.10	0.32	0.31	0.56	0.95	0.97
6	-0.32	0.26	0.00	0.00	0.14	0.37	0.39	0.62	1.34	1.16
7	-1.25	0.33	0.00	0.00	0.20	0.45	0.48	0.69	1.75	1.32
8	-2.30	0.25	0.53	0.73	0.14	0.37	0.12	0.35	1.29	1.14
9	-2.83	0.19	0.30	0.55	0.32	0.57	0.09	0.30	0.81	0.90
10	-2.96	0.26	0.65	0.81	0.03	0.17	0.28	0.53	0.55	0.74
End-systolic	-2.99	0.31	1.08	1.04	0.13	0.36	0.54	0.73	0.78	0.88

extracellular elastic domains are cyclically altered in diastole and systole, thereby leading to dynamic changes in local acoustic impedance mismatch.<sup>22</sup> However, other research workers found that changes in IB occur in isotonic but not isometric contraction of canine papillary muscle, suggesting that tissue elastic properties may be unimportant.<sup>24</sup> In a study of backscatter variables in the frequency domain, using dogs with open thorax, manipulation of heart rate, preload, and mean arterial pressure did not affect the backscatter variables, and the authors suggested that the cyclic variation was caused by an effective change in shape, area, and orientation of the scatterers within the myocardium.<sup>6</sup> Results of a recent study have suggested that cyclic variation in IB may be directly related to sarcomere length.<sup>25</sup>

The IB values in this study varied in magnitude corresponding with the cardiac cycle, in agreement with the findings in most other studies. The maximal value was found at or near the end-diastolic frame, and the minimal value was seen at or near the end-systolic frame. The amplitude of cyclic variation was also similar to the findings of other groups, as well as to other studies using this protocol.<sup>8,12</sup> Other authors have also reported that the left ventricular free wall has greater amplitude of cyclic variation than that of the interventricular septum.<sup>8</sup>

Calibration of backscatter measurements by use of a tissue phantom has been described by Yao et al.<sup>26</sup> Using the ratio of the signal from the canine scans and the phantom scans eliminates the effects of the transducer characteristics. Attenuation of the RF signal by the thoracic wall in dogs with closed thorax will inevitably vary between systole and diastole, thus affecting the results. However, the IB values reported in this study correspond with those previously reported in dogs with open thorax, suggesting that these effects are probably minimal.<sup>9-11</sup>

A potential problem associated with the described protocol is the use of a 5-MHz probe when digitization of the RF signal is carried out at 12 MHz, thus introducing the possibility of signal aliasing. However, a fourth-order Butterworth filter was used within the echocardiography machine to limit aliasing and reduce noise. Calculations derived from this system suggest that < 12% of the signal power is likely to be derived from the aliased signal. Another limitation of this protocol is the inability to acquire more than 15 frames/scan. As with nearly any other cardiac variable, some beat-to-beat variation is inevitable, and this study assessed as few as 2 cardiac cycles in some dogs. Despite this, the SD value for intradog variation was approximately 1 dB for end-diastole and end-systole, and was

negligible around early systole. The interdog variation was even less for end-diastole and end-systole (0.66 and 0.88 dB, respectively). Another disadvantage of this system is lack of an integrated timing reference, so that the simultaneous ECG was linked to the frames of the conventional scan, not to the specific RF data. The SD value for the end-diastolic to end-systolic amplitude of variation was large, although this may reflect the imprecise nature of the timing of individual frames, and does not take account of any phase delay of the cyclic variation.<sup>9</sup> However, the cyclic nature of the IB values was readily apparent (Fig 3), and this cyclic pattern would not be fully appreciated if analysis was limited to examination of end-diastolic and end-systolic frames only.

The advantage of the described protocol is the ability to collect RF data from the entire two-dimensional scan image. This provides freedom for the observer to choose specific regions of interest during subsequent off-line analysis, instead of limiting the data acquisition to regions predetermined at the time of scanning. The results suggest that the described protocol reliably indicates cyclic variation in integrated backscatter in normal, conscious dogs. Ultrasonic IB measurement offers the opportunity to measure myocardial tissue characteristics noninvasively, and has wide potential applications in the study of myocardial disease in small animals. Using the protocol described, this technique could be applied to clinical cases.

<sup>a</sup> ATL Ultramark 9 (UM9), Advanced Technology Laboratories, Bothell, Wash.

<sup>b</sup> Sparc System LX, Sun Microsystems Inc, Mountain View, Calif.

<sup>c</sup> PROC MIXED, SAS Institute Inc, Cary, NC.

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# Measurement of total body water content in horses, using deuterium oxide dilution

Frank M. Andrews, DVM, MS; Jenifer A. Nadeau, BS, MS; Lisa Saabye, BS; Arnold M. Saxton, PhD

**Objective**—To measure total body water (TBW) content in horses, using deuterium oxide ( $D_2O$ ) dilution.

**Animals**—Six 8- to 10-year-old healthy untrained mixed-breed horses, weighing (mean  $\pm$  SD)  $503.4 \pm 64.0$  kg.

**Procedure**—After a 12-hour nonfeeding period, 6 horses were given  $D_2O$  (0.14 g/kg of body weight) via nasogastric tube. Blood samples were collected from a preplaced indwelling jugular vein catheter prior to and 1 to 8, 10, 12, 14, and 24 hours after administration of  $D_2O$ . Blood samples were centrifuged immediately, and plasma was collected and stored at  $-70^\circ\text{C}$  until analysis. The  $D_2O$  content in plasma was measured by zinc reduction to deuterium gas. The resulting gas was measured, using an isotope ratio mass spectrometer.

**Results**—Deuterium oxide was rapidly absorbed from the gastrointestinal tract of all horses, and reached peak (mean  $\pm$  SD) plasma concentration ( $1,454.4 \pm 163$  delta D/ml or parts/thousand) 1 hour after administration. Plasma concentration decreased slowly during the next 2 to 3 hours, then remained statistically constant from 2 to 5 hours (early plateau phase) and 3 to 7 hours (late plateau phase) after administration. Mean  $\pm$  SEM TBW content was  $623.0 \pm 2.2$  ml/kg (62.3% of body weight) for the early plateau phase and  $630.3 \pm 2.2$  ml/kg (63.0% of body weight) for the late plateau phase.

**Conclusion**—Deuterium oxide dilution appears to be of value for measurement of TBW content in horses, and has a 4-hour plateau effect. Equilibration of  $D_2O$  with large intestinal water may be the reason for the prolonged equilibrium time and plateau effect seen in these horses.

**Clinical Relevance**—Deuterium oxide appears safe and efficacious for determining TBW content in horses and may be helpful for determining changes in TBW content during exercise and disease. (*Am J Vet Res* 1997;58:1060-1064)

**T**otal body water (TBW) content can change markedly in horses during exercise and in association with disease. Direct estimation of TBW content in animals and human beings requires administration of specific markers that distribute evenly in body water. Tritiated water and antipyrine have been used to estimate TBW content in horses, but these techniques are cumbersome, and in the case of tritiated water, expose horses and human beings to  $\beta$ -radiation.<sup>1-3</sup> On the other hand, deuterium oxide ( $D_2O$ ), a stable nonra-

dioactive isotope of water (heavy water), has been used to estimate TBW content in human beings,<sup>4-8</sup> pigs,<sup>9-10</sup> cattle,<sup>11-13</sup> lambs,<sup>14</sup> and dogs.<sup>15</sup> Deuterium oxide is preferred to other markers for measuring TBW content because it is similar to tap water, requires a small volume to be administered orally or IV, and is innocuous when given at these small volumes. The purpose of the study reported here was to evaluate the efficacy of a  $D_2O$  dilution technique for estimation of TBW content in untrained, nonfed horses at rest. Values obtained in these horses should serve as a baseline for comparison in the evaluation of fluid losses in horses during exercise and disease.

## Materials and Methods

**Animal model**—The following protocol was approved by the University of Tennessee College of Veterinary Medicine Animal Care and Use Committee (No. 715). Six healthy, 8- to 10-year-old, untrained, mixed-breed horses (1 gelding, 5 mares) were studied. Food was withheld for 12 hours prior to the start and for 8 hours during data collection. After the nonfeeding period, horses were given brome grass hay ad libitum. The horses were allowed ad libitum access to water during the study.

**Data collection**—Body weight was measured, using a portable walk-on scale,<sup>a</sup> which was equipped with load cell technology, had a weight range of 1.0 to 1,977 kg, was calibrated for linearity, and was accurate to within 1% of the measured weight. Deuterium oxide<sup>b</sup> (0.14 g/kg of body weight) was given via nasogastric tube at 8:00 AM. Blood samples were withdrawn from a preplaced jugular vein catheter into 5-ml evacuated tubes containing lithium heparin anticoagulant, before and 1 to 8, 10, 14, and 24 hours after  $D_2O$  administration. Blood samples were centrifuged immediately, and plasma was collected and stored at  $-70^\circ\text{C}$  until analysis.

**Deuterium oxide analysis**—The  $D_2O$  concentration of plasma was measured by zinc reduction at  $490^\circ\text{C}$  to produce deuterium gas. The resulting deuterium gas was measured by use of an isotope ratio mass spectrometer and expressed in parts per thousand or delta D/ml ( $\delta$ ) relative to Vienna standard mean ocean water (VSMOW),<sup>16</sup> which is the international reference standard for  $D_2O$ .

The TBW content (kg) was calculated, using the formula:

$$\frac{\text{Dose (g)} \times \text{APE}_{\text{dose}} \times 18.02 \text{ g/mole}}{\text{MW}_{\text{dose}} \times 100 \times (\delta_p - \delta_b) \times R_{\text{std}}}$$

where Dose = dose in grams,  $\text{APE}_{\text{dose}}$  = atom percent excess of dose (99.9%),  $\text{MW}_{\text{dose}}$  = molecular weight of  $D_2O$  = 20.02,  $\delta_p$  = delta D versus VSMOW for plateau sample,  $\delta_b$  = delta D of baseline sample (time 0), and  $R_{\text{std}}$  = ratio of deuterium to hydrogen in VSMOW (standard = 0.00015576).

**Statistical analysis**—Data were analyzed, using a general linear models procedure<sup>c</sup> to fit polynomial regression lines to each horse across time, and to compare regressions among horses. Terms up to the quadratic polynomial were tested, with the final model obtained by backward elimination of nonsignificant terms. A search was also made to find intervals of time when the regression lines were flat (slope

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From the Department of Large Animal Clinical Sciences (Andrews, Nadeau, Saabye), College of Veterinary Medicine, and Statistical and Computing Services, Agricultural Experiment Station (Saxton), the University of Tennessee, PO Box 1071, Knoxville, TN 37901-1071.

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